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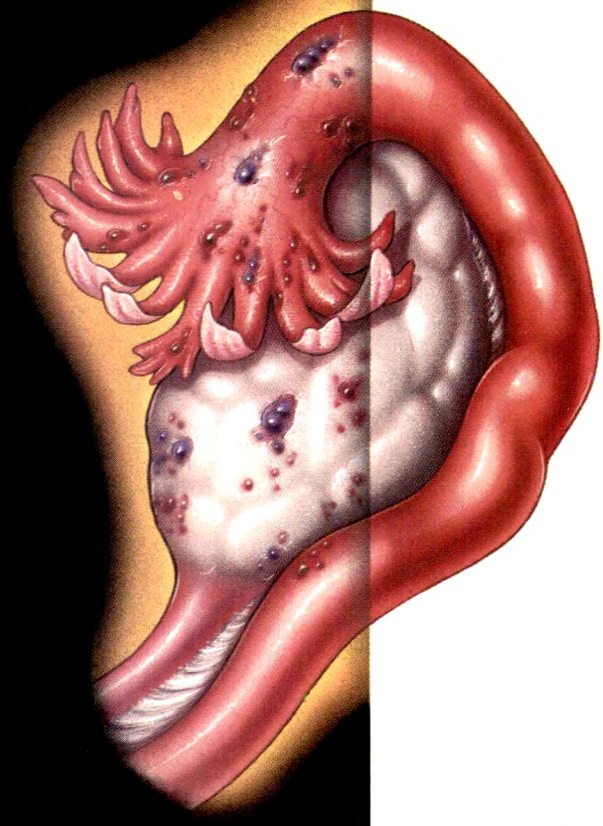
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IN THE MANAGEMENT OF ENDOMETRIOSIS



enough is enough

Power to suppress estradiol to levels that result in effective relief

- Lowers serum estradiol to near menopausal levels within approximately 1 month, and maintains those levels throughout the 6-month course of therapy.*
- 92% of patients (n = 143) were symptom-free or had only mild symptoms following a 6-month course of therapy.
- Retreatment cannot be recommended since safety data for retreatment are not currently available.

Control in the management of hypoestrogenic and therapeutic effec

- A small impact on bone,† as well as a predictable and manageable hypoestrogenic profile.
- Lets you terminate treatment immediately should that be required for any reason, unlike the depot form of GnRH‡ therapy.

*Pregnancy should be ruled out before therapy with SYNAREL is initiated, and patients should be advised to use a nonhormonal method of contraception when taking SYNAREL.
†There is a small loss in bone density with 6-month treatment, some of which may not be reversible. During one treatment period the bone loss should not be important. ‡Gonadotropin-releasing hormone analog. Please see brief summary of prescribing information on next page.



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NASAL SOLUTION, 2 MG/M

Setting The Pace In Endometriosis Therap

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PALO ALTO CA 94304

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BRIEF SUMMARY

SYNAREL® Nasal Solution contains nafarelin acetate (2 mg/mL, expressed as nafarelin base), benzalkonium chloride, glacial acetic acid, sodium hydroxide or hydrochloric acid, sorbitol, and purified water.

INDICATIONS AND USAGE: For management of endometriosis. Experience is limited to women 18 years and older treated for 6 months.

CONTRAINDICATIONS: Hypersensitivity to GnRH, GnRH agonist analogs or any excipients in SYNAREL; undiagnosed abnormal vaginal bleeding; pregnancy or possibility of pregnancy while taking the drug because SYNAREL may cause fetal harm when administered to a pregnant woman. If SYNAREL is used during pregnancy or if the patient becomes pregnant while taking this drug, she should be apprised of the potential hazard to the fetus; use in women who are breast feeding.

WARNINGS: Safe use in pregnancy has not been established clinically. Before starting treatment pregnancy must be excluded.

When used regularly at the recommended dose, SYNAREL usually inhibits ovulation and stops menstruation. Contraception is not insured, particularly if patients miss successive doses. Therefore, patients should use nonhormonal contraception. Advise patients to see their physician if they believe they may be pregnant. If a patient becomes pregnant during treatment, discontinue the drug; apprise patient of the potential risk to the fetus.

PRECAUTIONS General: Ovarian cysts have been reported in the first 2 months of therapy. These may resolve spontaneously or may require drug discontinuation or surgery.

Information for Patients: 1. Since menstruation should stop with SYNAREL, the patient should notify her physician if regular menstruation persists. If patient is compliant and menstruation persists to second month, consider doubling the dose. Counsel noncompliant patients on importance of taking SYNAREL regularly. 2. Patients should not use SYNAREL if pregnant, breast feeding, have undiagnosed abnormal vaginal bleeding, or are allergic to any of its ingredients. 3. Safe use in pregnancy has not been established. Therefore, a nonhormonal method of contraception should be used during treatment. If a patient becomes pregnant during treatment, she should discontinue the drug and consult her physician. 4. Adverse events occurring most frequently in clinical studies are associated with hypoestrogenism. Nasal irritation occurred in about 10% of patients. 5. Induced hypoestrogenic state results in a small loss in bone density over a 6-month treatment course. Some of this loss may not be reversible. During one six-month treatment period, this should not be important. In patients with major risk factors for decreased bone mineral content, i.e., chronic alcohol and/or tobacco use, strong family history of osteoporosis, or chronic use of drugs that can reduce bone mass such as anticonvulsants or corticosteroids, SYNAREL may pose additional risk. In these patients risks and benefits must be weighed carefully. Repeated courses of treatment with GnRH analogs are not advisable in patients with major risk factors for loss of bone mineral content. 6. Patients with intercurrent rhinitis should consult their physician for use of a topical nasal decongestant. If a topical nasal decongestant is required during treatment, it must be used at least 30 minutes after SYNAREL dosing to decrease the possibility of reducing drug absorption. 7. Retreatment cannot be recommended since safety data beyond 6 months are not available.

Drug Interactions: No pharmacokinetic-based drug-drug interaction studies have been conducted. Nafarelin acetate is a peptide primarily degraded by peptidase and not by cytochrome P-450 enzymes, and is only about 80% bound to plasma proteins at 4°C; therefore drug interactions would not be expected to occur.

Drug/Laboratory Test Interactions: SYNAREL in therapeutic doses suppresses the pituitary-gonadal system. Normal function is usually restored 4-8 weeks after treatment is discontinued. Therefore, diagnostic tests of pituitary gonadotropic and gonadal functions during treatment and up to 4-8 weeks after discontinuation of SYNAREL may be misleading.

Carcinogenesis, Mutagenesis, Impairment of Fertility: Carcinogenicity studies were conducted in rats and mice at intramuscular doses up to 110 times and 560 times the maximum recommended human intranasal dose, respectively. These multiples of the human dose are based on the relative bioavailability of the drug by the two routes of administration. As with other GnRH agonists, nafarelin acetate given to laboratory rodents at high doses for prolonged periods induced hyperplasia and/or neoplasia of endocrine organs. At 24 months, there was an increase in the incidence of pituitary tumors (adenoma/carcinoma) in high-dose female rats and a dose-related increase in male rats. There was an increase in pancreatic islet cell adenomas in both sexes, and in benign testicular and ovarian tumors in the treated groups. There was a dose-related increase in benign adrenal medullary tumors in treated female rats. In mice, there was a dose-related increase in Harderian gland tumors in males and an increase in pituitary adenomas in high-dose females. No metastases of these tumors were observed. It is known that tumorigenicity in rodents is particularly sensitive to hormonal stimulation.

Mutagenicity studies using bacterial, yeast, and mammalian systems provided no evidence of mutagenic potential.

Reproduction studies in male and female rats have shown full reversibility of fertility suppression when treatment was discontinued after continuous administration for up to 6 months.

Pregnancy, Teratogenic Effects: *Pregnancy Category X.* Intramuscular SYNAREL was administered to rats throughout gestation at about 0.5, 2, and 7 times the maximum recommended human intranasal dose based on the relative bioavailability by the two routes of administration. An increase in major fetal abnormalities was seen in 4/80 fetuses at the highest dose. A similar study at the same doses in rats and studies in mice and rabbits at doses up to 600 μg/kg/day and 0.18 μg/kg/day, respectively, failed to demonstrate an increase in fetal abnormalities after administration throughout gestation. In rats and rabbits, there was a dose-related increase in fetal mortality and a decrease in fetal weight with the highest dose.

Nursing Mothers: It is not known whether SYNAREL is excreted in human milk. Because many drugs are excreted in human milk, and because the effects of SYNAREL on lactation and/or the breastfed child have not been determined, SYNAREL should not be used by nursing mothers.

Pediatric Use: Safety and effectiveness in children have not been established.

ADVERSE REACTIONS: The most frequently reported adverse reactions were those related to hypoestrogenism. In controlled studies comparing SYNAREL (400 μg/day) and danazol (600 or 800 mg/day), the following adverse reactions were most frequently reported and thought to be drug-related.

Hypoestrogenic: hot flashes (SYNAREL 90%, danazol 69%), decreased libido (22%, 7%), vaginal dryness (19%, 7%), headaches (19%, 21%), emotional lability (15%, 18%), insomnia (8%, 4%); **Androgenic:** acne (13%, 20%), myalgia (10%, 23%), reduced breast size (10%, 16%), edema (8%, 23%), seborrhea (8%, 17%), weight gain (8%, 28%), hirsutism (2%, 6%), increased libido (1%, 6%); **Local:** nasal irritation (10%, 3%); **Miscellaneous:** depression (2%, 5%), weight loss (1%, 3%).

Less than 1% of patients experienced paresthesia, palpitations, chloasma, maculopapular rash, eye pain, urticaria, asthenia, lactation, breast engorgement, and arthralgia. In clinical trials, immediate hypersensitivity thought to be possibly or probably related to nafarelin occurred in 3 (0.2%) of 1509 healthy subjects or patients.

Changes in Bone Density: After 6 months' treatment vertebral trabecular bone density and total vertebral bone mass, measured by quantitative computed tomography (QCT), decreased by an average of 8.7% and 4.3%, respectively, compared to pretreatment levels. There was partial recovery of bone density post-treatment; the average trabecular bone density and total bone mass were 4.9% and 3.3% less than pretreatment levels, respectively. Total vertebral bone mass, measured by dual photon absorptiometry (DPA), decreased by a mean of 5.9% at end of treatment. Mean total vertebral mass, re-examined by DPA 6 months post-treatment, was 1.4% below pretreatment levels. There was little, if any, decrease in the mineral content in compact bone of the distal radius and second metacarpal. Use for longer than the recommended 6 months or in presence of other known risk factors for decreased bone mineral content may cause additional bone loss.

Changes in Laboratory Values: *Plasma enzymes.* During clinical trials SGOT and SGPT levels were more than twice the upper limit of normal in only one patient each. There was no other evidence of abnormal liver function and levels returned to normal after treatment was stopped.

Lipids. At enrollment and at end of treatment, 9% of patients on SYNAREL 400 μg/day and 2% on danazol had total cholesterol values above 250 mg/dL.

Of those with pretreatment cholesterol values below 250 mg/dL, 6% on SYNAREL and 18% on danazol had post-treatment values above 250 mg/dL.

Mean (±SEM) pretreatment values for total cholesterol from all patients were 191.8 (4.3) mg/dL in the SYNAREL group and 193.1 (4.6) mg/dL in the danazol group. At the end of treatment, mean values from all patients were 204.5 (4.8) mg/dL in the SYNAREL group and 207.7 (5.1) mg/dL in the danazol group. These increases from pretreatment values were significant ($p < 0.05$) in both groups.

Triglycerides were increased above the upper limit of 150 mg/dL in 12% of the patients on SYNAREL and in 7% of the patients on danazol.

At the end of treatment, no patients on SYNAREL had abnormally low HDL cholesterol fractions (< 30 mg/dL) compared with 43% of patients on danazol. No patients on SYNAREL had abnormally high LDL cholesterol fractions (> 190 mg/dL) compared with 15% of those on danazol. There was no increase in the LDL/HDL ratio in patients on SYNAREL, but approximately a 2-fold increase in the LDL/HDL ratio in patients on danazol.

Other changes. In comparative studies, the following changes were seen in approximately 10% to 15% of patients. SYNAREL was associated with elevations of plasma phosphorous and eosinophil counts, and decreases in serum calcium and WBC counts. Danazol therapy was associated with an increase of hematocrit and WBC.

OVERDOSAGE: At present there is no clinical evidence of adverse effects following overdosage of GnRH analogs.

SYNTEX

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April 1991
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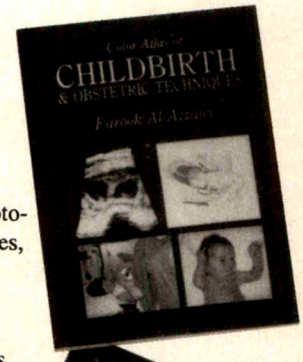
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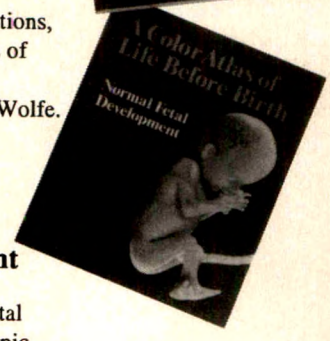
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F.A. Al-Azzawi, MBChB, MA, MRCOG



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*Please refer to product labeling for indicated organisms.
†In vitro activity does not necessarily correlate with in vivo effectiveness.

Please see adjacent page for brief summary of prescribing information.

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A BRIEF SUMMARY FOLLOWS.

INDICATIONS AND USAGE
TREATMENT

CEFOTAN is indicated for the therapeutic treatment of the following infections when caused by susceptible strains of the designated organisms:

Urinary Tract Infections caused by *E. coli, Klebsiella* species (including *K. pneumoniae*), *Proteus mirabilis*, and *Proteus* sp (which may include the organisms now called *Proteus vulgaris, Providencia rettgeri*, and *Morganella morganii*).

Lower Respiratory Tract Infections caused by *Streptococcus pneumoniae* (formerly *D. pneumoniae*), *Staphylococcus aureus* (penicillinase- and nonpenicillinase-producing strains), *Haemophilus influenzae* (including ampicillin-resistant strains), *Klebsiella* species (including *K. pneumoniae*), *E. coli, Proteus mirabilis*, and *Serratia marcescens**.

Skin and Skin Structure Infections due to *Staphylococcus aureus* (penicillinase- and nonpenicillinase-producing strains), *Staphylococcus epidermidis, Streptococcus pyogenes, Streptococcus* species (excluding enterococci), *Escherichia coli, Klebsiella pneumoniae, Peptococcus niger**, *Peptostreptococcus* species.

Gynecologic Infections caused by *Staphylococcus aureus* (including penicillinase- and nonpenicillinase-producing strains), *Staphylococcus epidermidis, Streptococcus* species (excluding enterococci), *Streptococcus agalactiae, E. coli, Proteus mirabilis, Neisseria gonorrhoeae,* Bacteroides species (excluding *B. distasonis, B. ovatus, B. thetaiotaomicron*), *Fusobacterium* species*, and gram-positive anaerobic cocci (including *Peptococcus niger* and *Peptostreptococcus* species).

Cefotetan, like other cephalosporins, has no activity against *Chlamydia trachomatis*. Therefore, when cephalosporins are used in the treatment of pelvic inflammatory disease, and *C. trachomatis* is one of the suspected pathogens, appropriate antichlamydial coverage should be added.

Intra-abdominal Infections caused by *E. coli, Klebsiella* species (including *K. pneumoniae*), *Streptococcus* species (excluding enterococci), *Bacteroides* species (excluding *B. distasonis, B. ovatus, B. thetaiotaomicron*) and *Clostridium* species*.

Bone and Joint Infections caused by *Staphylococcus aureus**.

*Efficacy for this organism in this organ system was studied in fewer than ten infections.

Specimens for bacteriological examination should be obtained in order to isolate and identify causative organisms and to determine their susceptibilities to cefotetan. Therapy may be instituted before results of susceptibility studies are known; however, once these results become available, the antibiotic treatment should be adjusted accordingly.

In cases of confirmed or suspected gram-positive or gram-negative sepsis or in patients with other serious infections in which the causative organism has not been identified, it is possible to use CEFOTAN concomitantly with an aminoglycoside. Cefotetan combinations with aminoglycosides have been shown to be synergistic *in vitro* against many Enterobacteriaceae and also some other gram-negative bacteria. The dosage recommended in the labeling of both antibiotics may be given and depends on the severity of the infection and the patient's condition.

NOTE: If CEFOTAN and an aminoglycoside are used concomitantly, renal function should be carefully monitored, especially if higher dosages of the aminoglycoside are to be administered or if therapy is prolonged, because of the potential nephrotoxicity and ototoxicity of aminoglycosidic antibiotics. Although, to date, nephrotoxicity has not been noted when CEFOTAN was given alone, it is possible that nephrotoxicity may be potentiated if CEFOTAN is used concomitantly with an aminoglycoside.

PROPHYLAXIS

The preoperative administration of CEFOTAN may reduce the incidence of certain postoperative infections in patients undergoing surgical procedures that are classified as clean contaminated or potentially contaminated (e.g., cesarean section, abdominal or vaginal hysterectomy, transurethral surgery, biliary tract surgery, and gastrointestinal surgery).

The prophylactic dose of CEFOTAN should be administered 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, CEFOTAN should be administered intravenously after the clamping of the umbilical cord.

If there are signs and symptoms of infection, specimens for culture should be obtained for identification of the causative organism so that appropriate therapeutic measures may be initiated.

CONTRAINDICATIONS

CEFOTAN is contraindicated in patients with known allergy to the cephalosporin group of antibiotics.

WARNINGS

BEFORE THERAPY WITH CEFOTAN IS INSTITUTED, CAREFUL INQUIRY SHOULD BE MADE TO DETERMINE WHETHER THE PATIENT HAS HAD PREVIOUS HYPERSENSITIVITY REACTIONS TO CEFOTETAN DISODIUM, CEPHALOSPORINS, PENICILLINS, OR OTHER DRUGS. IF THIS PRODUCT IS TO BE GIVEN TO PENICILLIN-SENSITIVE PATIENTS, CAUTION SHOULD BE EXERCISED BECAUSE CROSS-HYPERSENSITIVITY AMONG BETA-LACTAM ANTIBIOTICS HAS BEEN CLEARLY DOCUMENTED AND MAY OCCUR IN UP TO 10% OF PATIENTS WITH A HISTORY OF PENICILLIN ALLERGY. IF AN ALLERGIC REACTION TO CEFOTAN OCCURS, DISCONTINUE THE DRUG. SERIOUS ACUTE HYPERSENSITIVITY REACTIONS MAY REQUIRE TREATMENT WITH EPINEPHRINE AND OTHER EMERGENCY MEASURES, INCLUDING OXYGEN, INTRAVENOUS FLUIDS, INTRAVENOUS ANTIHISTAMINES, CORTICOSTEROIDS, PRESSOR AMINES AND AIRWAY MANAGEMENT, AS CLINICALLY INDICATED.

Pseudomembranous colitis has been reported with nearly all antibacterial agents, including cefotetan, and may range from mild to life-threatening. Onset of pseudomembranous colitis symptoms may occur during or after antibiotic treatment or surgical prophylaxis. Therefore, it is important to consider this diagnosis in patients who present with diarrhea subsequent to the administration of antibacterial agents.

Treatment with antibacterial agents alters the normal flora of the colon and may permit overgrowth of clostridia. Studies indicate that a toxin produced by *Clostridium difficile* is a primary cause of "antibiotic-associated colitis."

After the diagnosis of pseudomembranous colitis has been established, therapeutic measures should be initiated. Mild cases of pseudomembranous colitis usually respond to discontinuation of the drug alone. In moderate to severe cases, consideration should be given to management with fluids and electrolytes, protein supplementation, and treatment with an oral antibacterial drug effective against *Clostridium difficile*.

In common with many other broad-spectrum antibiotics, CEFOTAN may be associated with a fall in prothrombin activity and, possibly, subsequent bleeding. Those at increased risk include patients with renal or hepatobiliary impairment or poor nutritional state, the elderly, and patients with cancer. Prothrombin time should be monitored and exogenous vitamin K administered as indicated.

PRECAUTIONS

General: As with other broad-spectrum antibiotics, prolonged use of CEFOTAN may result in overgrowth of nonsusceptible organisms. Careful observation of the patient is essential. If superinfection does occur during therapy, appropriate measures should be taken.

CEFOTAN should be used with caution in individuals with a history of gastrointestinal disease, particularly colitis.

Information for Patients: As with some other cephalosporins, a disulfiram-like reaction characterized by flushing, sweating, headache, and tachycardia may occur when alcohol (beer, wine, etc.) is ingested within 72 hours after CEFOTAN administration. Patients should be cautioned about the ingestion of alcoholic beverages following the administration of CEFOTAN.

Drug Interactions: Although to date nephrotoxicity has not been noted when CEFOTAN was given alone, it is possible that nephrotoxicity may be potentiated if CEFOTAN is used concomitantly with an aminoglycoside.

Drug/Laboratory Test Interactions: The administration of CEFOTAN may result in a false-positive reaction for glucose in the urine using Clinitest®†, Benedict's solution, or Fehling's solution. It is recommended that glucose tests based on enzymatic glucose oxidase be used.

As with other cephalosporins, high concentrations of cefotetan may interfere with measurement of serum and urine creatinine levels by Jaffe reaction and produce false increases in the levels of creatinine reported.

Carcinogenesis, Mutagenesis, Impairment of Fertility: Although long-term studies in animals have not been performed to evaluate carcinogenic potential, no mutagenic potential of cefotetan was found in standard laboratory tests.

Cefotetan has adverse effects on the testes of prepubertal rats. Subcutaneous administration of 500 mg/kg/day (approximately 8-16 times the usual adult human dose) on days 6-35 of life (thought to be developmentally analogous to late childhood and prepuberty in humans) resulted in reduced testicular weight and seminiferous tubule degeneration in 10 of 10 animals. Affected cells included spermatogonia and spermatocytes; Sertoli and Leydig cells were unaffected. Incidence and severity of lesions were dose-dependent; at 120 mg/kg/day (approximately 2-4 times the usual human dose) only 1 of 10 treated animals was affected, and the degree of degeneration was mild.

Similar lesions have been observed in experiments of comparable design with other methylthiotetrazole-containing antibiotics and impaired fertility has been reported, particularly at high dose levels. No testicular effects were observed in 7-week-old rats treated with up to 1000 mg/kg/day SC for 5 weeks, or in infant dogs (3 weeks old) that received up to 300 mg/kg/day IV for 5 weeks. The relevance of these findings to humans is unknown.

Pregnancy: Teratogenic Effects. Pregnancy Category B: Reproduction studies have been performed in rats and monkeys at doses up to 20 times the human dose and have revealed no evidence of impaired fertility or harm to the fetus due to cefotetan. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproductive studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Nursing Mothers: Cefotetan is excreted in human milk in very low concentrations. Caution should be exercised when cefotetan is administered to a nursing woman.

Pediatric Use: Safety and effectiveness in children have not been established.

ADVERSE REACTIONS

In clinical studies, the following adverse effects were considered related to CEFOTAN therapy. Those appearing in italics have been reported during postmarketing experience.

Gastrointestinal symptoms occurred in 1.5% of patients; the most frequent were diarrhea (1 in 80) and nausea (1 in 700); *pseudomembranous colitis*. Onset of pseudomembranous colitis symptoms may occur during or after antibiotic treatment or surgical prophylaxis. (See WARNINGS.)

Hematologic laboratory abnormalities occurred in 1.4% of patients and included eosinophilia (1 in 200), positive direct Coombs' test (1 in 250), and thrombocytosis (1 in 300); *agranulocytosis, hemolytic anemia, leukopenia, thrombocytopenia,* and *prolonged prothrombin time*.

Hepatic enzyme elevations occurred in 1.2% of patients and included a rise in SGPT (1 in 150), SGOT (1 in 300), alkaline phosphatase (1 in 700), and LDH (1 in 700).

Hypersensitivity reactions were reported in 1.2% of patients and included rash (1 in 150) and itching (1 in 700); *anaphylactic reactions*.

Local effects were reported in less than 1.0% of patients and included phlebitis at the site of injection (1 in 300), and discomfort (1 in 500).

Miscellaneous: *Fever*

In addition to the adverse reactions listed above which have been observed in patients treated with cefotetan, the following adverse reactions and altered laboratory tests have been reported for cephalosporin-class antibiotics: urticaria, pruritis, Stevens-Johnson syndrome, erythema multiforme, toxic epidermal necrolysis, vomiting, abdominal pain, colitis, superinfection, vaginitis including vaginal candidiasis, renal dysfunction, toxic nephropathy, hepatic dysfunction including cholestasis, aplastic anemia, hemorrhage, increased BUN, increased creatinine, elevated bilirubin, pancytopenia, and neutropenia.

Several cephalosporins have been implicated in triggering seizures, particularly in patients with renal impairment when the dosage was not reduced (see DOSAGE AND ADMINISTRATION and OVERDOSAGE). If seizures associated with drug therapy occur, the drug should be discontinued. Anticonvulsant therapy can be given if clinically indicated.

DOSAGE AND ADMINISTRATION
TREATMENT

The usual adult dosage is 1 or 2 grams of CEFOTAN administered intravenously or intramuscularly every 12 hours for 5 to 10 days. Proper dosage and route of administration should be determined by the condition of the patient, severity of the infection, and susceptibility of the causative organism.

GENERAL GUIDELINES FOR DOSAGE OF CEFOTAN

Type of Infection	Daily Dose	Frequency and Route
Urinary Tract	1-4 grams	500 mg every 12 hours IV or IM 1 or 2 g every 24 hours IV or IM 1 or 2 g every 12 hours IV or IM
Skin & Skin Structure		
Mild-Moderate[a]	2 grams	2 g every 24 hours IV 1 g every 12 hours IV or IM
Severe	4 grams	2 g every 12 hours IV
Other Sites	2-4 grams	1 or 2 g every 12 hours IV or IM
Severe	4 grams	2 g every 12 hours IV
Life-Threatening	6 grams[b]	3 g every 12 hours IV

[a] *K. pneumoniae* skin and skin structure infections should be treated with 1 or 2 grams every 12 hours IV or IM.

[b] Maximum daily dosage should not exceed 6 grams.

If *C. trachomatis* is a suspected pathogen in gynecologic infections, appropriate antichlamydial coverage should be added, since cefotetan has no activity against this organism.

PROPHYLAXIS

To prevent postoperative infection in clean contaminated or potentially contaminated surgery in adults, the recommended dosage is 1 or 2 g of CEFOTAN administered once, intravenously, 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, the dose should be administered as soon as the umbilical cord is clamped.

IMPAIRED RENAL FUNCTION

When renal function is impaired, a reduced dosage schedule must be employed. See FULL PRESCRIBING INFORMATION for a complete description.

NOTE: Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration whenever solution and container permit.

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References: **1.** Walker CK, Landers DV, Ohm-Smith MJ, et al. Comparison of cefotetan plus doxycycline with cefoxitin plus doxycycline in the inpatient treatment of acute salpingitis. *Sex Trans Dis.* 1991;18:119-123. **2.** McGregor JA, Gordon SF, Krotec J, Poindexter AN. Results of a randomized, multicenter, comparative trial of a single dose of cefotetan versus multiple doses of cefoxitin as prophylaxis in cesarean section. *Am J Obstet Gynecol.* 1988;158:701-706. **3.** Carver P, Quintiliani R, Nightingale CH. Comparative pharmacokinetic study of cefotetan and cefoxitin in healthy volunteers. *Infect Surg.* April 1986(suppl):11-14. **4.** Ohm-Smith MJ, Sweet RL, Hadley WK. In vitro activity of cefbuperazone and other antimicrobial agents against isolates from the female genital tract. *Antimicrob Agents Chemother.* 1985;27:958-960. **5.** Data on file, Stuart Pharmaceuticals, Wilmington, Delaware. **6.** Souza Dias MB, Jacobus NV, Tally FP, Gorbach SL. Activity of cefotetan against anaerobic bacteria. *Diagn Microbiol Infect Dis.* 1986;4:359-363. **7.** Wexler HM, Finegold SM. In vitro activity of cefotetan compared with that of other antimicrobial agents against anaerobic bacteria. *Antimicrob Agents Chemother.* 1988;32:601-604. **8.** Hennessey TD. Cefotetan: a compilation of susceptibility data of clinical isolates. In: Lode H, Periti P, Strachan CJL, eds. *Cefotetan: A Long-Acting Antibiotic for Polymicrobial Infections.* New York, NY: Churchill Livingstone; 1985:14-22. **9.** Centers for Disease Control. 1989 sexually transmitted diseases treatment guidelines. *MMWR.* 1989;38(suppl S-8):1-43.

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Atlanta, Georgia

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Buserelin acetate versus expectant management in the treatment of infertility associated with minimal or mild endometriosis: A randomized clinical trial 1345
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Münster, Germany

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Burlington, Vermont

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Gungor Eroglu, MD, George Betz, MD, and Caprice Torregano, BS
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Boston, Massachusetts

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New Haven and Danbury, Connecticut, and Detroit, Michigan

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Houston, Texas

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Ultrastructure of the microvasculature in the human endometrium throughout the normal menstrual cycle 1393
Daniel K. Roberts, MD, PhD, Tim H. Parmley, MD, Nola J. Walker, EMSA, and Douglas V. Horbelt, MD
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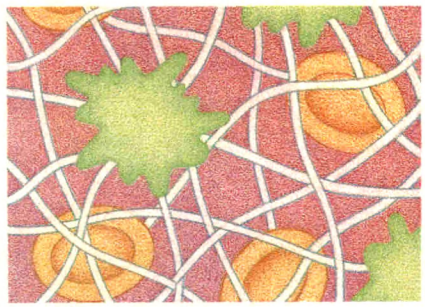
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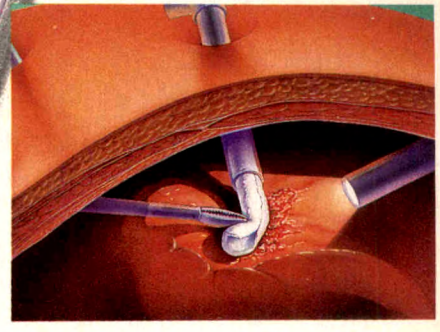
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Baltimore, Maryland

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Infertility and pregnancy outcome in an unselected group of women with insulin-dependent diabetes mellitus 1412
Karen Kjaer, MD, Claus Hagen, MD, Steen H. Sandø, MD, and Ole Eshøj, MD
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Utrecht, The Netherlands

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Sharon H. Nelson, PhD, and Maya S. Suresh, MD
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Levonorgestrel and ethinyl estradiol tablets—Triphasic regimen

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TRI-LEVLEN® 28 TRI-LEVLEN® 21

BRIEF SUMMARY

Tri-Levlen®—6 brown tablets, each containing 0.050 mg of levonorgestrel (d(-)-13 beta-ethyl-17-alpha-ethinyl-17-beta-hydroxygon-4-en-3-one), a totally synthetic progestogen, and 0.030 mg of ethinyl estradiol (19-nor-17α-pregna-1,3,5(10)-trien-20-yne-3, 17-diol); 5 white tablets, each containing 0.075 mg levonorgestrel and 0.040 mg ethinyl estradiol; 10 light-yellow tablets, each containing 0.125 mg levonorgestrel and 0.030 mg ethinyl estradiol (7 light-green tablets containing inert ingredients are included in the 28-day triphasic regimen).

Indications and Usage

Oral contraceptives are indicated for the prevention of pregnancy in women who elect to use this product as a method of contraception.

Contraindications

Oral contraceptives should not be used in women with any of the following conditions: thrombophlebitis or thromboembolic disorders, a past history of deep-vein thrombophlebitis or thromboembolic disorders, cerebral-vascular or coronary-artery disease, known or suspected carcinoma of the breast, carcinoma of the endometrium or other known or suspected estrogen-dependant neoplasia, undiagnosed abnormal genital bleeding, cholestatic jaundice of pregnancy or jaundice with prior pill use, hepatic adenomas or carcinomas, known or suspected pregnancy.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral-contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risks of several serious conditions including myocardial infarction, thromboembolism, stroke, hepatic neoplasia, gallbladder disease, and hypertension, although the risk of serious morbidity or mortality is very small in healthy women without underlying risk factors. The risk of morbidity and mortality increases significantly in the presence of other underlying risk factors such as hypertension, hyperlipidemias, obesity and diabetes. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks. The information contained in this package insert is principally based on studies carried out in patients who used oral contraceptives with higher formulations of estrogens and progestogens than those in common use today. The effect of long-term use of the oral contraceptives with lower formulations of both estrogens and progestogens remains to be determined. Throughout this labeling, epidemiological studies reported are of two types: retrospective or case control studies and prospective or cohort studies. Case control studies provide a measure of the relative risk of disease, namely, a ratio of the incidence of a disease among oral-contraceptive users to that among nonusers. The relative risk does not provide information on the actual clinical occurrence of a disease. Cohort studies provide a measure of attributable risk, which is the difference in the incidence of disease between oral-contraceptive users and nonusers. The attributable risk does provide information about the actual occurrence of a disease in the population. For further information, the reader is referred to a text on epidemiological methods.

1. *Thromboembolic Disorders and Other Vascular Problems*

a. *Myocardial Infarction.* An increased risk of myocardial infarction has been attributed to oral-contraceptive use. This risk is primarily in smokers or women with other underlying risk factors for coronary-artery disease such as hypertension, hypercholesterolemia, morbid obesity, and diabetes. The relative risk of heart attack for current oral-contraceptive users has been estimated to be two to six. The risk is very low under the age of 30. Smoking in combination with oral-contraceptive use has been shown to contribute substantially to the incidence of myocardial infarctions in women in their mid-thirties or older with smoking accounting for the majority of excess cases. Mortality rates associated with circulatory disease have been shown to increase substantially in smokers over the age of 35 and non-smokers over the age of 40 among women who use oral contraceptives. Oral contraceptives may compound the effects of well-known risk factors, such as hypertension, diabetes, hyperlipidemias, age and obesity. In particular, some progestogens are known to decrease HDL cholesterol and cause glucose intolerance, while estrogens may create a state of hyperinsulinism. Oral contraceptives have been shown to increase blood pressure among users (see section 9 in "Warnings"). Similar effects on risk factors have been associated with an increased risk of heart disease. Oral contraceptives must be used with caution in women with cardiovascular disease risk factors.

b. *Thromboembolism.* An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Case control studies have found the relative risk of users compared to nonusers to be 3 for the first episode of superficial venous thrombosis, 4 to 11 for deep-vein thrombosis or pulmonary embolism, and 1.5 to 6 for women with predisposing conditions for venous thromboembolic disease. Cohort studies have shown the relative risk to be somewhat lower, about 3 for new cases and about 4.5 for new cases requiring hospitalization. The risk of thromboembolic disease due to oral contraceptives is not related to length of use and disappears after pill use is stopped. A two-to four-fold increase in relative risk of postoperative thromboembolic complications has been reported with the use of oral contraceptives. The relative risk of venous thrombosis in women who have predisposing conditions is twice that of women without such medical conditions. If feasible, oral contraceptives should be discontinued at least four weeks prior to and for two weeks after elective surgery of a type associated with an increase in risk of thromboembolism and during and following prolonged immobilization. Since the immediate post partum period is also associated with an increased risk of thromboembolism, oral contraceptives should be started no earlier than four to six weeks after delivery in women who elect not to breast-feed, or a midtrimester pregnancy termination.

c. *Cerebrovascular diseases.* Oral contraceptives have been shown to increase both the relative and attributable risks of cerebrovascular events (thrombotic and hemorrhagic strokes), although, in general, the risk is greatest among older (>35 years), hypertensive women who also smoke. Hypertension was found to be a risk factor for both users and nonusers, for both types of strokes, while smoking interacted to increase the risk for hemorrhagic strokes. In a large study, the relative risk of thrombotic strokes has been shown to range from 3 for normotensive users to 14 for users with severe hypertension. The relative risk of hemorrhagic stroke is reported to be 1.2 for nonsmokers who used oral contraceptives, 2.6 for smokers who did not use oral contraceptives, 7.6 for smokers who used oral contraceptives, 1.8 for normotensive users and 25.7 for users with severe hypertension. The attributable risk is also greater in older women.

d. *Dose-related risk of vascular disease from oral contraceptives.* A positive association has been observed between the amount of estrogen and progestogen in oral contraceptives and the risk of vascular disease. A decline in serum high-density lipoproteins (HDL) has been reported with many progestational agents. A decline in serum high-density lipoproteins has been associated with an increased incidence of ischemic heart disease. Because estrogens increase HDL cholesterol, the net effect of an oral contraceptive depends on a balance achieved between doses of estrogen and progestogen and the nature and absolute amount of progestogen used in the contraceptive. The amount of both hormones should be considered in the choice of an oral contraceptive. Minimizing exposure to estrogen and progestogen is in keeping with good principles of therapeutics. For any particular estrogen/progestogen combination, the dosage regimen prescribed should be one which contains the least amount of estrogen and progestogen that is compatible with a low failure rate and the needs of the individual patient. New acceptors of oral-contraceptive agents should be started on preparations containing less than 50 mcg of estrogen.

e. *Persistence of risk of vascular disease.* There are two studies which have shown persistence of risk of vascular disease for ever-users of oral contraceptives. In a study in the United States, the risk of developing myocardial infarction after discontinuing oral contraceptives persists for at least 9 years for women 40-49 years who had used oral contraceptives for five or more years, but this increased risk was not demonstrated in other age groups. In another study in Great Britain, the risk of developing cerebrovascular disease persisted for at least 6 years after discontinuation of oral contraceptives, although excess risk was very small. However, both studies were performed with oral-contraceptive formulations containing 50 micrograms or higher of estrogens.

2. *Estimates of Mortality from Contraceptive Use.* One study gathered data from a variety of sources which have estimated the mortality rate associated with different methods of contraception at different ages. These estimates include the combined risk of death associated with contraceptive methods plus the risk attributable to pregnancy in the event of method failure. Each method of contraception has its specific benefits and risks. The study concluded that with the exception of oral-contraceptive users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of birth control is less than that associated with childbirth. The observation of a possible increase in risk of mortality with age for oral-contraceptive users is based on data gathered in the 1970's–but not reported until 1983. However, current clinical practice involves the use of lower estrogen dose formulations combined with careful restriction of oral-contraceptive use to women who do not have the various risk factors listed in this labeling. Because of these changes in practice and, also, because of some limited new data which suggest that the risk of cardiovascular disease with the use of oral contraceptives may now be less than previously observed, the Fertility and Maternal Health Drugs Advisory Committee was asked to review the topic in 1989. The Committee concluded that although cardiovascular-disease risks may be increased with oral-contraceptive use after age 40 in healthy nonsmoking women (even with the newer low-dose formulations), there are greater potential health risks associated with pregnancy in older women and with the alternative surgical and medical procedures which may be necessary if such women do not have access to effective and acceptable means of contraception. Therefore, the Committee recommended that the benefits of oral-contraceptive use by healthy nonsmoking women over 40 may outweigh the possible risks. Of course, older women, as all women who take oral contraceptives, should take the lowest possible dose formulation that is effective.

3. *Carcinoma of the Reproductive Organs.* Numerous epidemiological studies have been performed on the incidence of breast, endometrial, ovarian and cervical cancer in women using oral contraceptives. The overwhelming evidence in the literature suggests that use of oral contraceptives is not associated with an increase in the risk of developing breast cancer, regardless of the age and parity of first use or with most of the marketed brands and doses. The Cancer and Steroid Hormone (CASH) study also showed no latent effect on the risk of breast cancer for at least a decade following long-term use. A few studies have shown a slightly increased relative risk of developing breast cancer, although the methodology of these studies, which included differences in examination of users and nonusers and differences in age at start of use, has been questioned. Some studies suggest that oral-contraceptive use has been associated with an increase in the risk of cervical intraepithelial neoplasia in some populations of women. However, there continues to be controversy about the extent to which such findings may be due to differences in sexual behavior and other factors. In spite of many studies of the relationship between oral-contraceptive use and breast and cervical cancers, a cause-and-effect relationship has not been established.

4. *Hepatic Neoplasia.* Benign hepatic adenomas are associated with oral-contraceptive use, although the incidence of benign tumors is rare in the United States. Indirect calculations have estimated the attributable risk to be in the range of 3.3 cases/100,000 for users, a risk that increases after four or more years of use. Rupture of rare, benign, hepatic adenomas may cause death through intra-abdominal hemorrhage. Studies from Britain have shown an increased risk of developing hepatocellular carcinoma in long-term (>8 years) oral-contraceptive users. However, these cancers are extremely rare in the U.S. and the attributable risk (the excess incidence) of liver cancers in oral-contraceptive users approaches less than one per million users.

5. *Ocular Lesions.* There have been clinical case reports of retinal thrombosis associated with the use of oral contraceptives. Oral contraceptives should be discontinued if there is unexplained partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions. Appropriate diagnostic and therapeutic measures should be undertaken immediately.

6. *Oral-Contraceptive Use Before or During Early Pregnancy.* Extensive epidemiological studies have revealed no increased risk of birth defects in women who have used oral contraceptives prior to pregnancy. Studies also do not suggest a teratogenic effect, particularly insofar as cardiac anomalies and limb-reduction defects are concerned, when taken inadvertently during early pregnancy. The administration of oral contraceptives to induce withdrawal bleeding should not be used as a test for pregnancy. Oral contraceptives should not be used during pregnancy to treat threatened or habitual abortion. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing oral-contraceptive use. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period. Oral-contraceptive use should be discontinued if pregnancy is confirmed.

7. *Gallbladder Disease.* Earlier studies have reported an increased lifetime relative risk of gallbladder surgery in users of oral contraceptives and estrogens. More recent studies, however, have shown that the relative risk of developing gallbladder disease among oral-contraceptive users may be minimal. The recent findings of minimal risk may be related to the use of oral-contraceptive formulations containing lower hormonal doses of estrogens and progestogens.

8. *Carbohydrate and Lipid Metabolic Effects.* Oral contraceptives have been shown to cause glucose intolerance in a significant percentage of users. Oral contraceptives containing greater than 75 micrograms of estrogens cause hyperinsulinism, while lower doses of estrogen cause less glucose intolerance. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents. However, in the nondiabetic woman, oral contraceptives appear to have no effect on fasting blood glucose. Because of these demonstrated effects, prediabetic and diabetic women should be carefully observed while taking oral contraceptives. A small proportion of women will have persistent hypertriglyceridemia while on the pill. As discussed earlier (see "Warnings" 1a. and 1d.), changes in serum triglycerides and lipoprotein levels have been reported in oral-contraceptive users.

9. *Elevated Blood Pressure.* An increase in blood pressure has been reported in women taking oral contraceptives and this increase is more likely in older oral-contraceptive users and with continued use. Data from the Royal College of General Practitioners and subsequent randomized trials have shown that the incidence of hypertension increases with increasing quantities of progestogens. Women with a history of hypertension or hypertension-related diseases, or renal disease should be encouraged to use another method of contraception. If women with hypertension elect to use oral contraceptives, they should be monitored closely, and if significant elevation of blood pressure occurs, oral contraceptives should be discontinued. For most women, elevated blood pressure will return to normal after stopping oral contraceptives, and there is no difference in the occurrence of hypertension among ever-and never-users.

10. *Headache.* The onset or exacerbation of migraine or development of headache with a new pattern that is recurrent, persistent or severe requires discontinuation of oral contraceptives and evaluation of the cause.

11. *Bleeding Irregularities.* Breakthrough bleeding and spotting are sometimes encountered in patients on oral contraceptives, especially during the first three months of use. The type and dose of progestogen may be important. Nonhormonal causes should be considered and adequate diagnostic measures taken to rule out malignancy or pregnancy in the event of breakthrough bleeding, as in the case of any abnormal vaginal bleeding. If pathology has been excluded, time or a change to another formulation may solve the problem. In the event of amenorrhea, pregnancy should be ruled out. Some women may encounter post-pill amenorrhea or oligomenorrhea, especially when such a condition was preexistent.

Precautions

1. PHYSICAL EXAMINATION AND FOLLOW UP. A complete medical history and physical examination should be taken prior to the initiation or reinstitution of oral contraceptives and at least annually during use of oral contraceptives. These physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including cervical cytology, and relevant laboratory tests. In case of undiagnosed, persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be conducted to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care. 2. LIPID DISORDERS. Women who are being treated for hyperlipidemias should be followed closely if they elect to use oral contraceptives. Some progestogens may elevate LDL levels and may render the control of hyperlipidemias more difficult. (See "Warnings" 1d.) 3. LIVER FUNCTION. If jaundice develops in any woman receiving such drugs, the medication should be discontinued. Steroid hormones may be poorly metabolized in patients with impaired liver function. 4. FLUID RETENTION. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention. 5. EMOTIONAL DISORDERS. Patients becoming significantly depressed while taking oral contraceptives should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug related. Women with a history of depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. 6. CONTACT LENSES. Contact-lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist. 7. DRUG INTERACTIONS. Reduced efficacy and increased incidence of breakthrough bleeding and menstrual irregularities have been associated with concomitant use of rifampin. A similar association, though less marked, has been suggested with barbiturates, phenylbutazone, phenytoin sodium, and possibly with griseofulvin, ampicillin and tetracyclines. 8. INTERACTIONS WITH LABORATORY TESTS. Certain endocrine-and liver-function tests and blood components may be affected by oral contraceptives: a. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. b. Increased thyroid-binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. c. Other binding proteins may be elevated in serum. d. Sex-binding globulins are increased and result in elevated levels of total circulating sex steroids and corticoids; however, free or biologically active levels remain unchanged. e. Triglycerides may be increased. f. Glucose tolerance may be decreased. g. Serum folate levels may be depressed by oral-contraceptive therapy. This may be of clinical significance if a woman becomes pregnant shortly after discontinuing oral contraceptives. 9. CARCINOGENESIS. See "Warnings" section. 10. PREGNANCY. Pregnancy Category X. See "Contraindications" and "Warnings" sections. 11. NURSING MOTHERS. Small amounts of oral-contraceptive steroids have been identified in the milk of nursing mothers and a few adverse effects on the child have been reported, including jaundice and breast enlargement. In addition, oral contraceptives given in the postpartum period may interfere with lactation by decreasing the quantity and quality of breast milk. If possible, the nursing mother should be advised not to use oral contraceptives but to use other forms of contraception until she has completely weaned her child.

Information for the Patient

See Patient Labeling.

Adverse Reactions

An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see "Warnings" section): thrombophlebitis, arterial thromboembolism, pulmonary embolism, myocardial infarction, cerebral hemorrhage, cerebral thrombosis, hypertension, gallbladder disease, hepatic adenomas or benign liver tumors.

There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: mesenteric thrombosis, retinal thrombosis.

The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug related: nausea; vomiting; gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding; spotting; change in menstrual flow; amenorrhea; temporary infertility after discontinuation of treatment; edema; melasma which may persist; breast changes: tenderness, enlargement, secretion; change in weight (increase or decrease); change in cervical erosion and secretion; diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening); intolerance to contact lenses.

The following adverse reactions have been reported in users of oral contraceptives and the association has been neither confirmed nor refuted: congenital anomalies, premenstrual syndrome, cataracts, optic neuritis, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, cerebral-vascular disease with mitral valve prolapse, lupus-like syndromes, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, impaired renal function, hemolytic uremic syndrome, Budd-Chiari syndrome, acne, changes in libido, colitis, sickle-cell disease.

Overdosage

Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Noncontraceptive Health Benefits

The following noncontraceptive health benefits related to the use of oral contraceptives are supported by epidemiological studies which largely utilized oral-contraceptive formulations containing doses exceeding 0.035 mg of ethinyl estradiol or 0.05 mg of mestranol.

Effects on menses: increased menstrual cycle regularity, decreased blood loss and decreased incidence of iron-deficiency anemia, decreased incidence of dysmenorrhea.

Effects related to inhibition of ovulation: decreased incidence of functional ovarian cysts, decreased incidence of ectopic pregnancies.

Effects from long-term use: decreased incidence of fibroadenomas and fibrocystic disease of the breast, decreased incidence of acute pelvic inflammatory disease, decreased incidence of endometrial cancer, decreased incidence of ovarian cancer.

Dosage and Administration

To achieve maximum contraceptive effectiveness, TRI-LEVLEN® tablets (Levonorgestrel and Ethinyl Estradiol Tablets—Triphasic Regimen) must be taken exactly as directed and at intervals not exceeding 24 hours.

For full details on dosage and administration see prescribing information in package insert. 60627-0 5/90

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Los Angeles, California

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Carl P. Weiner, MD
Iowa City, Iowa

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Göttingen, Martinsried, Munich, and Bielefeld, Germany, and Debrecen, Hungary

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Houston, Texas

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Los Angeles, California

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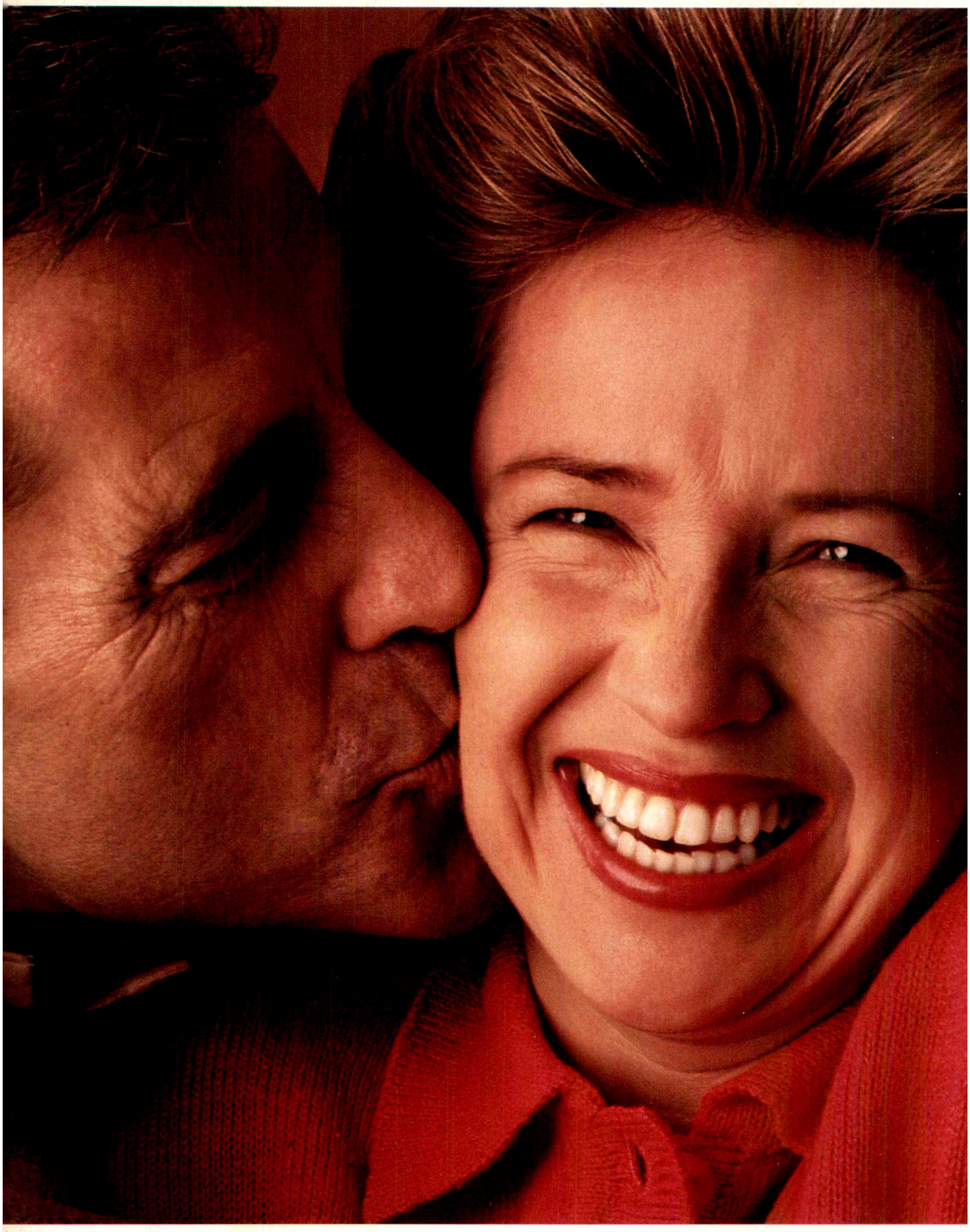
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Contraindications

Estrogens should not be used in women (or men) with any of the following conditions: known or suspected 1) pregnancy, 2) breast cancer, 3) estrogen-dependent neoplasia, 4) undiagnosed abnormal genital bleeding, 5) active thrombophlebitis or thromboembolic disorders.

Note: Estrogens have been reported to increase the risk of endometrial carcinoma in postmenopausal women.



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Please see brief summary of prescribing information on next page.

BRIEF SUMMARY *(FOR FULL PRESCRIBING INFORMATION AND PATIENT INFORMATION, SEE PACKAGE CIRCULARS.)*

PREMARIN® Brand of conjugated estrogens tablets, USP
PREMARIN® Brand of conjugated estrogens Vaginal Cream, in a nonliquefying base

1. ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA. Close clinical surveillance of all women taking estrogens is important. Adequate diagnostic measures including endometrial sampling when indicated should be undertaken to rule out malignancy in all cases of undiagnosed persistent or recurring abnormal vaginal bleeding. There is currently no evidence that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.
2. ESTROGENS SHOULD NOT BE USED DURING PREGNANCY.
Estrogen therapy during pregnancy is associated with an increased risk of congenital defects in the reproductive organs of the male and female fetus, an increased risk of vaginal adenosis, squamous-cell dysplasia of the uterine cervix, and vaginal cancer in the female later in life. The 1985 DES Task Force concluded that women who used DES during their pregnancies may subsequently experience an increased risk of breast cancer. However, a causal relationship is still unproven, and the observed level of risk is similar to that for a number of other breast cancer risk factors.
There is no indication for estrogen therapy during pregnancy. Estrogens are ineffective for the prevention or treatment of threatened or habitual abortion.

DESCRIPTION: PREMARIN (conjugated estrogens, USP) contains a mixture of estrogens, obtained exclusively from natural sources, blended to represent the average composition of material derived from pregnant mares' urine. It contains estrone, equilin, and 17α-dihydroequilin, together with smaller amounts of 17α-estradiol, equilenin, and 17α-dihydroequilenin as salts of their sulfate esters. Tablets are available in 0.3 mg, 0.625 mg, 0.9 mg, 1.25 mg, and 2.5 mg strengths of conjugated estrogens. Cream is available as 0.625 mg conjugated estrogens per gram.

INDICATIONS AND USAGE: Moderate-to-severe vasomotor symptoms associated with the menopause. (There is no evidence that estrogens are effective for nervous symptoms or depression which might occur during menopause and they should not be used to treat these conditions.) Prevention and management of osteoporosis (abnormally low bone mass). Atrophic vaginitis. Atrophic urethritis. Hypoestrogenism due to hypogonadism, castration or primary ovarian failure.

PREMARIN (conjugated estrogens) Vaginal Cream is indicated in the treatment of atrophic vaginitis and kraurosis vulvae.

PREMARIN HAS NOT BEEN SHOWN TO BE EFFECTIVE FOR ANY PURPOSE DURING PREGNANCY AND ITS USE MAY CAUSE SEVERE HARM TO THE FETUS (SEE BOXED WARNING).

CONTRAINDICATIONS: Estrogens should not be used in women (or men) with any of the following conditions: 1. Known or suspected pregnancy (see Boxed Warning). 2. Known or suspected cancer of the breast except in appropriately selected patients being treated for metastatic disease. 3. Known or suspected estrogen-dependent neoplasia. 4. Undiagnosed abnormal genital bleeding. 5. Active thrombophlebitis or thromboembolic disorders. 6. Estrogen replacement therapy has not been reported to increase the risk of thrombophlebitis and/or thromboembolic disease. However, there is insufficient information regarding women who have had previous thromboembolic disease.

PREMARIN Tablets and Vaginal Cream should not be used in patients hypersensitive to their ingredients.

WARNINGS: Some studies suggest a possible increased incidence of breast cancer in women taking higher doses of estrogen for prolonged time periods. The majority of studies have not shown an association with usual estrogen replacement doses. Endometrial cancer risk among estrogen users was about 4-fold or greater than in non-users, and appears dependent on treatment duration and estrogen dose. In patients on combined estrogen-progestin therapy, this risk appears to be decreased. (See PRECAUTIONS below.)

Estrogen therapy during pregnancy is associated with an increased risk of fetal congenital reproductive tract disorders.

A 2.5-fold increase in the risk of surgically confirmed gall bladder disease in women receiving postmenopausal estrogens has been reported.

Large doses of estrogen such as those used to treat prostate and breast cancer have been shown to increase the risk of non-fatal myocardial infarction, pulmonary embolism, and thrombophlebitis in men. This cannot necessarily be extrapolated to women. However, to avoid theoretical cardiovascular risk caused by high estrogen doses, the doses for estrogen replacement therapy should not exceed the recommended dose.

Blood pressure should be monitored with estrogen use, especially if high doses are used.

Estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases.

PRECAUTIONS: The addition of a progestin for 7 or more days of a cycle of estrogen administration reportedly lowers the incidence of endometrial hyperplasia. Studies of endometrium suggest that 10 to 13 days of progestin are needed to provide maximal endometrial maturation and elimination of hyperplastic changes. Additional risks, such as adverse effects on carbohydrate and lipid metabolism, may be associated with the inclusion of progestin in estrogen replacement regimens. The choice of progestin and dosage may be important in minimizing these adverse effects.

Physical examination and a complete medical and family history should be taken prior to the initiation of any estrogen therapy with special reference to blood pressure, breasts, abdomen, and pelvic organs, and should include a Papanicolaou smear. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination being performed. Conditions influenced by fluid retention, such as asthma, epilepsy, migraine, and cardiac or renal dysfunction, require careful observation. Certain patients may develop manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding and mastodynia. Pre-existing uterine leiomyomata may increase in size during estrogen use. Estrogens should be used with care in patients with impaired liver function, renal insufficiency, or metabolic bone diseases associated with hypercalcemia.

The following drug/laboratory test interactions have been reported, some only with estrogen-progestin combinations (oral contraceptives):

1. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.
2. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by T_4 levels determined by column or by radioimmunoassay. Free T_3 resin uptake is decreased, reflecting the elevated TBG; free T_4 concentration is unaltered.
3. Impaired glucose tolerance.
4. Reduced response to metyrapone test.
5. Reduced serum folate concentration.

MUTAGENESIS AND CARCINOGENESIS: Long-term, continuous administration of natural and synthetic estrogens in certain animal species increases the frequency of carcinomas of the breast, cervix, vagina, and liver.

PREGNANCY CATEGORY X: Estrogens should not be used during pregnancy. See **CONTRAINDICATIONS** and Boxed Warning.

NURSING MOTHERS: As a general principle, the administration of any drug to nursing mothers should be done only when clearly necessary since many drugs are excreted in human milk.

ADVERSE REACTIONS: The following have been reported with estrogenic therapy: changes in vaginal bleeding pattern and abnormal withdrawal bleeding or flow, breakthrough bleeding, spotting, increase in size of uterine fibromyomata, vaginal candidiasis, change in amount of cervical secretion; tenderness or enlargement of breasts; nausea, vomiting, abdominal cramps, bloating, cholestatic jaundice; chloasma or melasma that may persist when drug is discontinued, erythema multiforme, erythema nodosum, hemorrhagic eruption, loss of scalp hair, hirsutism; steepening of corneal curvature, intolerance to contact lenses; headache, migraine, dizziness, mental depression, chorea; increase or decrease in weight; reduced carbohydrate tolerance; aggravation of porphyria; edema; changes in libido.

ACUTE OVERDOSAGE: May cause nausea and vomiting.

DOSAGE AND ADMINISTRATION:
PREMARIN® Brand of conjugated estrogens tablets, USP

1. *Given cyclically for short-term use only.* For treatment of moderate-to-severe vasomotor symptoms, atrophic vaginitis, or atrophic urethritis associated with the menopause (0.3 mg to 1.25 mg or more daily). The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible. Administration should be cyclic (eg, three weeks on and one week off). Attempts to discontinue or taper medication should be made at three- to six-month intervals.
2. *Given cyclically:* Hypoestrogenism. Osteoporosis.
Hypoestrogenism due to: Female hypogonadism—2.5 mg to 7.5 mg daily in divided doses for 20 days followed by 10 day rest period. If bleeding does not occur by the end of this period, the same dosage schedule is repeated. Female castration or primary ovarian failure—1.25 mg daily, cyclically. Adjust upward or downward according to response of the patient. For maintenance, adjust dosage to lowest level that will provide effective control.
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Reference: 1. Weiss NS, Ure CL, Ballard JH, et al: Decreased risk of fractures of the hip and lower forearm with postmenopausal use of estrogen. *N Engl J Med* 1980;303:1195-1198.

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Manuscript submission. Manuscripts should be submitted to one of the two Editors in Chief as follows:

1. **Dr. Zuspan**—manuscripts from the eastern and southeastern United States and from England, Israel, and Italy, manuscripts written for Clinical Opinion and Current Development, Letters to the Editors, and all manuscripts presented before one of the official sponsoring societies, except the Society for Gynecologic Investigation. *Manuscripts from The Society of Perinatal Obstetricians should be submitted to Dr. J. Peter Van Dorsten, P.O. Box 34, MCV Station, Richmond, VA 23298.*
2. **Dr. Quilligan**—manuscripts from states west of Pennsylvania, West Virginia, Kentucky, Tennessee, and Mississippi; manuscripts from Hawaii, Alaska, Canada, and abroad (except England, Israel, and Italy). Dr. Quilligan is responsible for manuscripts from the Society for Gynecologic Investigation. *Manuscripts from the Society for Gynecologic Investigation should be submitted to Dr. Roger A. Lobo, Women's Hospital, 1240 North Mission Road, Room 1M2, Los Angeles, CA 90033.*

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Previous publication. If a report by the same author(s) or some of the same authors has been previously published in any medium that deals in any respect whatsoever with the same patients, same animals, same laboratory experiments, or same data, in part or in full, as those reported in the manuscript being submitted, two reprints of the article(s) or two copies of the manuscript, be it a full-length report or an abstract, must be submitted with the manuscript. Two copies of a new manuscript being prepared for possible publication also must be submitted. The author(s) should inform the Editor of the circumstances, similarities, and differences of the reports. This requirement also applies to the submission of a manuscript in which a few different patients, animals, laboratory experiments, or data were added to those reported in a previous publication or in a submitted or accepted manuscript. Articles previously published in another language will not be considered.

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The original and two good-quality photocopies of the manuscript and three sets of black-and-white glossy prints of illustrations are required. See "Color figures" and "Computer-generated figures" for special requirements. Manuscripts must be submitted in English.

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Abstract page including key words/phrases. On manuscript page 3, type the abstract, double-spaced, with the required margins and headed by the title of the article and name(s) of author(s). Below the abstract, list 3 to 5 *key words or short phrases* for indexing purposes.

A structured abstract is required for certain types of articles and a *standard abstract format* is required for other types of articles; therefore the author(s) should select and prepare the appropriate abstract to be used in the submitted manuscript. This applies to both independently submitted and society manuscripts.

Structured abstract. A structured abstract, limited to 150 words, is to be used for regular articles, society articles, and Current Investigation articles; excluded are Clinical Opinion, Current Development, case report, and brief communication articles. The structured abstract is to contain the following major headings: *Objective(s); Study Design; Results;* and *Conclusion(s).* The *Objective(s)* reflects the purpose of the study, that is, the hypothesis that is being tested. The *Study Design* should include the setting for the study, the subjects (number and type), the treatment or intervention, and the type of statistical analysis. The *Results* include the outcome of the study and statistical significance if appropriate. The *Conclusion(s)* states the significance of the results.

Standard abstract. The standard abstract format is required for Clinical Opinion, Current Development, case report, and brief communication articles. The standard abstracts for Clinical Opinion and Current Development articles are to be 50 to 150 words and standard abstracts for case reports and brief communications are limited to 50 words. Even though the formal names of these four types of articles are not used, an author should determine his/her type of manuscript and prepare the appropriate standard abstract, regardless of whether it is to be submitted independently or as a part of a society's transactions.

Text. Do not hesitate to write your manuscript in the first person and active voice if they are more appropriate to the information you wish to convey. The passive voice is generally more effective for describing techniques or observations, since the emphasis is on the "action" rather than on the person performing the action.

Only standard abbreviations are to be used. Consult the

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With the exception of Current Development articles, the length of text material (introduction through *Comment* section) in regular manuscripts accepted for publication normally ranges from 750 to 4200 words (an average of 2000 words). A text of 4200 words or more can seldom be accepted, especially if tables and figures are included. The average manuscript of 2000 words of text with abstract, 3 tables with captions, 2 figures with legends, and references makes a 5.7-page article in the JOURNAL. The 2000 words of text alone makes approximately 8 pages of manuscript typed double-spaced with the required 1-inch margins (approximately 250 words per page). A table or figure that occupies both columns of half a JOURNAL page is equivalent to approximately 500 words in manuscript. Thus, if a greater number of illustrations and tables is used, the length of the text should be adjusted accordingly.

Council of Biology Editors Style Manual or the AMA's *Manual of Style.* Abbreviations in the title are not acceptable. They should be avoided, if possible, in the abstract. In the text they should be kept to a practical minimum. The full term for which an abbreviation stands should precede its first use in the text unless it is a standard unit of measurement.

Either the generic, chemical, or proprietary names of drugs may be used. If the generic or chemical name is used, authors may, if they desire, insert the proprietary name in parentheses after the first mention in the text, with the name of the manufacturer and city and state.

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Examples (if six or fewer authors, list all; if seven or more authors, list three then et al.):

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CLINICAL SECTION

Clinical Articles

Resident attrition in obstetrics and gynecology

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OBJECTIVE: Our goal was to determine the rate of attrition from obstetrics and gynecology residency programs.

STUDY DESIGN: The Council on Resident Education in Obstetrics and Gynecology sent questionnaires to all 295 obstetrics and gynecology residency program directors in the United States and Canada. These programs represent 4306 postgraduate-year 1 through 4 (or 5) resident positions each year. The program directors were asked the number of residents who left voluntarily or were dismissed in a 2-year period and the reasons they left.

RESULTS: In a 2-year period 299 residents left or were dismissed (6.94% over 2 years, or 3.47% per year). Only 88 (1% per year) left specifically because they decided they preferred a different discipline.

CONCLUSION: The rate of attrition from obstetrics and gynecology residency programs is not excessively high. (AM J OBSTET GYNECOL 1992;166:1315-7.)

Key words: Resident education, resident dismissal, resident attrition

In the 1990 National Residents' Matching Program, obstetrics and gynecology was the most popular of the six major specialties. The Program filled 86% of the obstetrics and gynecology positions with U.S. medical school seniors on the first round of the match, compared with 67% for general surgery, 63% for pediatrics, and 61% for internal medicine. When the Program was completed only 3% of obstetrics and gynecology positions were vacant, compared with 21% for internal medicine and 30% for family practice.

Of the 1044 postgraduate year 1 positions for obstetrics and gynecology residencies starting in 1990 entered through the Program, 925 were filled with U.S. medical school seniors, 51 with U.S. medical school graduates who did not go immediately into obstetrics and gynecology, 4 with Fifth Pathway students, 8 with U.S. foreign medical school graduates, 25 with alien foreign medical graduates, 30 with doctors of osteopathy, and 1 with a Canadian medical school graduate. In addition, approximately 100 positions were filled outside the match, 60 from armed forces programs. In 1990 there were approximately 170 senior American medical students who wanted obstetrics and gynecology as their specialty but were not matched with a position; in 1989 there were 80 and in 1988 52.

There are more American medical school graduates interested in careers in obstetrics and gynecology than there are positions, and there is concern that there will be increasing shortages of obstetrician-gynecologists in several regions of the country[1]; therefore, it is important to assess whether the medical students who obtain residency positions in our discipline actually complete the residency or whether there is an excessive attrition rate.

The Council on Resident Education in Obstetrics and Gynecology therefore decided to determine the attrition rate and cause in our residency programs.

Material and methods

At the request of the Council on Resident Education in Obstetrics and Gynecology (CREOG), in 1989 one of the authors (R.M.) sent a survey to each of the 295 obstetrics and gynecology residency program directors in the United States and Canada asking how many residents were currently in their programs, how many had left during the past 2 years, and the reason the residents left. These data were tabulated by the CREOG office.

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Table I. Reasons for leaving obstetrics and gynecology residencies during a 2-year period

Reason	*No.*
Training in another specialty	88
Training in another obstetrics and gynecology residency program	62
Dismissal from program	58
Spouse relocation	40
Miscellaneous	51
TOTAL	299

Table II. Number of residents leaving a program during a 2-year period

No. of residents leaving	*No. of programs*	*Total residents leaving*
0	109	0
1	87	87
2	40	80
3	20	60
4	9	36
5	6	30
6	1	6
TOTAL		299

Results

Of the 295 surveys sent, 265 replies were received (90% response rate) from program directors responsible for 4306 postgraduate year 1 to postgraduate year 4 (or 5) residents per year. On the 265 replies, 117 were from obstetrics and gynecology residency programs within medical schools, 114 from programs affiliated with medical schools, and 34 from institutions with no relationship to a medical school.

During the 2-year period for which data were requested, 299 residents left. Table I indicates the reasons residents left obstetrics and gynecology programs. Eighty-eight residents left to train in another specialty, choosing a variety of fields including pediatrics, internal medicine, family practice, anesthesia, psychiatry, radiation therapy, and surgery. Reasons cited for these changes to other disciplines included stress, demanding work hours, inability to fulfill commitment, unsuitable personality, and disenchantment with the specialty.

Sixty-two residents left to train in other obstetrics and gynecology residencies. Of those residents, 35 were originally from university programs, 16 from medical school–affiliated programs, and 11 from programs not affiliated with a medical school.

Fifty-eight residents were dismissed and 40 residents left their programs because of spouse relocation. The 51 residents who left for miscellaneous reasons, cited issues such as finances, personal illness, family illness, and drug use. In five instances residents left their programs because of issues relating to maternity or child care. We do not know whether residents who left because of spouse relocation, miscellaneous reasons, or dismissal ultimately reentered obstetrics and gynecology programs or residency programs in other disciplines or did not reenter residencies at all.

Table II indicates the number of residents leaving each institution. Nine programs each had 4 residents leave the program. Three of these 9 programs each had only 8 residents, 1 had 10 residents, 2 had 16, 1 had 20, 1 had 21, and 1 had 44 residents. Six programs each had 5 residents leaving the program. One of these programs had 8 residents, 1 had 10, 1 had 11, 1 had 12, and the other 2 had 24 residents each. One 13-resident program had 6 leave.

Comment

There is a growing concern that there will continue to be an increasing shortage of obstetricians and gynecologists to meet the health care needs of American women.[1] Therefore it is important that medical students are carefully counseled in career decisions by informed members of their medical schools. In addition to the internal resources of individual departments and medical schools, more formal programs for career specialty decisions may be available. One such program known to us is the Glaxo pathway evaluation program, which helps students to identify their personal and career goals. In addition to the guidance given to students by their medical schools, the residency selection process should make certain that those individuals selected will have the motivation and capability to complete the residency program and meet the health care, education, and research needs of our discipline.

Ninety percent of programs surveyed by the Council responded, reporting that 299 residents left their programs in a 2-year period (3.47% per year). Only 88 residents left programs specifically because they wished to train in another specialty (1% per year). This suggests that medical school departments of obstetrics and gynecology are providing appropriate counseling and exposure so that the medical students have a reasonable appreciation of the discipline and what the demands and rewards of a residency in this field entail. Sixty-two residents (0.72% per year) left to train in other obstetrics and gynecology residencies. In many instances there were several residents leaving the same program but continuing training in the field. Our survey was not detailed enough to enable us to evaluate why this occurred, but it is likely that in some instances there may have been sudden disruptions in a program. In 40 instances (0.46% per year) residents left programs because of spouse relocation. This is a surprisingly small percentage given the numbers of residents who have spouses with careers and the forced mobility this sometimes entails. Also of note is that only five instances

were reported in a 2-year period in which residents left because of issues relating to maternity or child care. Because approximately 47% of obstetrics and gynecology residents are women and almost 50% of married female residents become pregnant during their residency,[2] this is a very small number.

Fifty-eight residents (0.67%) were dismissed. Program directors and residency selection committees are continually refining their selection processes in an attempt to select the most promising residents. In spite of strict selection criteria it is often difficult to identify the types of problems that ultimately lead to resident dismissal.

In conclusion we feel that 3.47% per year attrition from our programs is not excessive, particularly because many of these residents are not leaving the discipline of obstetrics and gynecology, but rather are changing from one program to another. However, we should continue to reevaluate and refine student career counseling and resident selection processes to keep resident attrition from our discipline and residency programs at a low rate.

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Repeated fetal losses associated with antiphospholipid antibodies: A collaborative randomized trial comparing prednisone with low-dose heparin treatment

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OBJECTIVE: We attempted to compare the use of low-dose heparin with a standard dose of 40 mg prednisone daily (both plus low-dose aspirin) for treatment of pregnant women with antiphospholipid antibody–associated recurrent fetal loss with respect to maternal and perinatal morbidity and efficacy in prevention of fetal death.

STUDY DESIGN: A multicenter randomized trial included 20 patients. Generalizability of results from randomized patients was evaluated by means of additional data from 13 women refusing and 12 women ineligible for randomization. Data from study groups were compared with Fisher's exact test, and generalizability was evaluated with a χ^2 test for trend.

RESULTS: Live birth rates were the same (75%) with either treatment, but "serious" maternal morbidity and the frequency of preterm delivery were significantly higher among women randomly assigned to prednisone ($p = 0.02$ vs $p = 0.006$). Preterm delivery among prednisone-treated women was usually associated with premature rupture of the membranes or preeclampsia. These results could be generalized to the other groups of women ascertained during the course of the study.

CONCLUSIONS: Low-dose heparin should be preferred to prednisone when treatment is indicated for high-risk pregnant women with antiphospholipid antibodies. (Am J Obstet Gynecol 1992;166:1318-23.)

Key words: Heparin, prednisone, antiphospholipic antibodies

The term antiphospholipid antibodies refers to a broad group of autoantibodies including both lupus anticoagulants and antibodies to cardiolipin. Antiphospholipid antibodies have been associated with a history of fetal loss and thrombosis in women with or without systemic lupus erythematosus.[1-7] The predictive value of antiphospholipid antibody tests for fetal death or thrombosis in varied groups of women, with or without a history of fetal loss or treatment, ranges from 48% to 76%.[3, 4, 7] When antiphospholipid antibodies are present, continuing pregnancies may be complicated by thrombosis, preeclampsia, fetal distress, preterm delivery, or low birth weight.[3, 7-9] Successful treatment of such pregnancies with 40 to 60 mg prednisone and 80 mg aspirin daily was first reported by Lubbe et al.[10] in 1983. Rates of live births with treatment, usually aspirin and prednisone, have averaged 60% to 76%.[5, 6]

The morbidity associated with prednisone treatment led to consideration of anticoagulation with heparin to prevent both thrombosis and fetal death in affected pregnancies. The rationale for the use of heparin was the observation of infarction in placentas of affected pregnancies and reports that lupus anticoagulants may interfere with the activation of protein C by thrombin bound to thrombomodulin.[11] Heparin may compensate for deficient levels of protein C(a), or activated protein C, which inactivates clotting factors Va and VIIIa. Heparin was given in anticoagulant doses without aspirin, or in prophylactic doses with low-dose aspirin, in both individual case reports and in the first published series of cases.[12, 13] Rates of live births in the latter were equal to those reported for prednisone-treated women, but both series included some women with only one previous fetal death. Only women with histories of at least two prior fetal losses associated with a confirmed diagnosis of antiphospholipid antibodies were included in this prospective study. We compared pregnancy outcomes after treatment with aspirin plus either prednisone or prophylactic doses of heparin in the first collaborative trial of therapies for affected women.

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Material and methods

Eligibility criteria and randomization. All subjects had two or more prior unexplained fetal losses. The diagnosis of an antiphospholipid antibody required two or more positive tests (lupus anticoagulant or anticardiolipin antibodies) over at least a 6-week period. Other accepted causes for recurrent miscarriage or fetal death were excluded. Randomization was performed centrally with a computer-generated sequence of random numbers after pregnancy was confirmed. Written consent, approved by institutional review boards at each investigating center, was obtained from every participant. Women were excluded from randomized treatment assignment when a relative contraindication or indication for the use of one of the treatment drugs was documented. Twelve women were ineligible because of a history of diabetes ($n = 2$), a history of thrombosis outside of pregnancy ($n = 2$), current treatment with prednisone for another indication ($n = 3$), previous fetal loss with one of the treatments (three of four were previously treated with prednisone), or thrombocytopenia ($n = 1$). An additional 13 women refused randomized treatment assignment. Data from patients not randomly assigned to a treatment were collected during the course of the study to investigate possible selection bias and generalizability of results from randomly treated women.

Treatment protocols. Aspirin (80 mg daily) was prescribed for all patients and given throughout pregnancy. Prednisone was given as soon as pregnancy was confirmed, in divided doses of 20 mg every 12 hours. Prednisone doses were lowered in two cases to improve control of insulin-dependent diabetes in the third trimester. Heparin was initiated at a dose of 10,000 U self-administered subcutaneously every 12 hours as soon as a potentially viable pregnancy was documented by ultrasonography (6.5 to 8 weeks). Subsequent doses were adjusted downward by 2000 U until a midinterval activated partial thromboplastin time was within the normal range or similar to prolonged baseline values. The mean daily dose was 17,000 U, similar to dosages for pregnant women on the basis of measurement of the activation of factor X.[15] Two women in the heparin treatment group miscarried before ultrasonographic documentation of pregnancy viability and therefore were not treated. Heparin treatment was not begun until ectopic pregnancy could be excluded by ultrasonography. All women given heparin were also given vitamin D (600 to 800 U) and calcium carbonate (4 gm). Similar supplementation was not advised for women receiving prednisone because corticosteroids primarily affect bone collagen matrix.

Data collection and statistical analysis. Patients came from seven participating centers. The study was initiated in March 1988 and continued through September 1990. Enrollment data included obstetric and medical histories, and the results of a complete blood count, creatinine, antinuclear antibody titer, levels of serum complement, and antiphospholipid antibody test results. The American Rheumatism Association criteria were used for the diagnosis of systemic lupus erythematosus.[16]

Enzyme-linked immunosorption assays for immunoglobulin M and immunoglobulin G class anticardiolipin antibodies were performed at collaborating institutions and repeated on at least one aliquot of serum in a central laboratory according to a previously described method.[17] Positive results were confirmed if the value after subtraction of nonspecific binding fell at or above 2 multiples of the median for a normal obstetric population. These cutoff values were equivalent to >30 GPL (IgG phospholipid units) and >11 MPL (IgM phospholipid units) units using commercially available kits (REAADS Medical Products, Inc., and Kallestad Diagnostics Inc.). All patients had anticardiolipin antibody levels equivalent to or higher than the midpositive range of Harris et al.[18] Tests for lupus anticoagulants included activated partial thromboplastin time (45% of cases) or dilute Russell viper venom time performed on a fresh or fresh-frozen plasma sample in a central laboratory.[19] Abnormal coagulation times (2 SD above the mean) were confirmed by 1 : 1 dilution of the sample with fresh-frozen plasma to exclude clotting factor deficiencies.

Outcome data included medical and obstetric complications. Fetal distress was diagnosed on the basis of nonreassuring fetal heart rate monitoring with or without abnormal ultrasonographic findings (biophysical profile). Deliveries before 37 completed postmenstrual weeks were considered preterm. Low birth weight was defined as birth weight below the 10th percentile for gestational age. We excluded the following complications unrelated to antiphospholipid antibodies or treatment from analysis: two fetal losses (one in patient with incompetent cervix given heparin and one with fetal aneuploidy given prednisone) and two elective preterm deliveries in the heparin group (single cases of discordant twins and placenta previa).

Maternal morbidity was tabulated for those who completed at least 6 weeks of treatment. Morbidity was classified as minor (problems such as thrush or transient nose bleeds), manageable (problems requiring treatment such as gestational diabetes), or serious (potentially life-threatening or with permanent sequelae such as maternal preeclampsia with both hypertension and proteinuria and one case of posterior capsular cataracts).

Statistical analyses. Differences in rates and means between groups were compared with Fisher's exact test (two-tailed) and a standard t test. Generalizability of

Table I. Comparison of study patients ($N = 20$) by treatment group

Characteristic	*Heparin (n = 12)*	*Prednisone (n = 8)*
Obstetric history		
Mean No. of spontaneous abortions	2.8	2.9
No. with at least 1 or ≥2 losses after 12 wk	6/12 (50%)	3/8 (38%)
≥3 Abortions, all before 12 wk	3/12 (25%)	3/8 (38%)
2 Abortions before 12 wk*	3/12 (25%)	2/8 (25%)
Uncomplicated live birth without treatment within previous 5 yr	0/12	0/8
Antiphospholipid antibodies		
Lupus anticoagulant present	4/12 (33%)	3/8 (38%)
IgM anticardiolipin antibody only	2/12 (17%)	1/8 (13%)
Live births in prospectively treated pregnancy	9/12 (75%)	6/8 (75%)
Live births excluding those with just two abortions before 12 wk	6/9 (67%)	4/6 (67%)
Fetal deaths after 12 wk	1/12 (8%)	2/8 (25%)

*Only one woman in whom antiphospholipid antibodies were restricted to IgM class anticardiolipin antibody binding was among those with a history of just two miscarriages before 12 weeks.

results from study patients to the other groups of women was tested with a χ^2 test for trend. The likelihood of a false-negative error and power analysis for sample sizes were calculated with published tables and nomograms.[20,21]

Previous studies suggested that the rate of live births would be approximately 50% for heparin-treated women if treatment were ineffective. The study was continued until statistically significant differences in maternal or fetal morbidity or mortality with either treatment were evident on yearly interim data analysis.

Results

Interim analysis of pregnancy outcomes for 20 women who were eligible for and accepted random assignment to one of the treatments by the end of the second study year is reported here. Equal numbers of women were not assigned to each treatment at this point because allocation was to be balanced when the total reached 50. Obstetric histories, pertinent laboratory studies, and pregnancy outcomes after treatment are summarized in Table I. Women randomly assigned to a treatment had two to four (mean of three) sequential previous abortions. Most (15/20) had at least one fetal death after 12 weeks or had had three or more spontaneous abortions in the first trimester. None had historic or laboratory findings satisfying three or more of the American Rheumatism Association criteria for the diagnosis of systemic lupus erythematosus or an uncomplicated live birth without treatment in the 5 years before the treated pregnancy. Treatment groups were similar with respect to medical and obstetric histories and laboratory results ($p = 0.7$ to 1.0). The frequency of live birth after treatment was 75% in each group. The 95% confidence intervals for these figures were 43% to 95% (heparin group) and 35% to 97% (prednisone group). Live birth rates are also shown after exclusion of women with just two first-trimester miscarriages. This number of randomly assigned patients did not have the power (alpha = 0.05, beta = 0.80) to exclude a 50% difference with respect to rates of live births.

In contrast to the similar live birth rates in the two treatment groups, women randomly assigned to prednisone were significantly more likely to be delivered preterm (6/6 vs 2/8, $p = 0.006$). Preterm delivery was associated with premature rupture of the membranes (3/6 vs 0.9, $p = 0.04$) or preeclampsia (Table II). Preterm delivery was an important problem for infants of prednisone-treated women; on average they were delivered 5 weeks earlier than those in the heparin group (mean of 32 vs 37 postmenstrual weeks, $p = 0.05$).

Maternal morbidity is also shown in Table II for all women treated for at least 6 weeks, including three whose pregnancies ended in fetal death. In contrast to those treated with heparin, women given prednisone were significantly more likely to have a serious complication (preeclampsia and one case of posterior capsular cataracts) ($p = 0.02$) and often had more than one complication.

Selection bias among randomized women was evaluated by comparing study women with women ascertained during the course of the study who refused or were not eligible for randomization (Table III). The eligibility criteria tended to exclude women with systemic lupus erythematosus or "lupus-like" disorders. Thus women not eligible for randomized assignment because of thrombocytopenia or concurrent treatment with prednisone were more likely to have at least three criteria for the diagnosis of systemic lupus erythematosus ($p = 0.01$, compared with randomized women). All women with a history of a fetal death with treatment (usually prednisone) were also assigned to this group. For these reasons ineligible women were probably at higher prior risk for fetal death than the study group. Eligible women refusing randomization were more likely to have an uncomplicated live birth within the

Table II. Obstetric and medical complications among women with live births (n = 15) or fetal death after 12 weeks (n = 3) who were treated with prednisone or heparin

Complication	*Prednisone*	*Heparin*	*p Value*
Obstetric			
No.	6	9	
Preterm delivery	6/6	2/8*	0.006
Premature rupture of membranes	3/6	0/9	0.04
Mean week at delivery	32	37	0.05
Maternal			
No.	8	10	
Minor			
Thrush	2/8		
Bleeding†		2/10	
Superficial thrombosis		1/10	
	1.0		
Manageable			
Diabetes	3/8	1/10	0.3
Serious			
Preeclampsia	3/8	0/10	0.02
Cataracts	1/8		
Any maternal morbidity	6/8 (75%)	4/10 (40%)	0.2

*Case of discordant twins was excluded from calculation.

†Nosebleeds (n = 1) and postpartum vaginal bleeding (n = 1); neither required treatment.

preceding 5 years (p = 0.05). Therefore they may have been at lower prior risk for fetal death than the study group. In spite of these potentially important differences in medical and obstetric histories, women who refused and ineligible women had rates of live births (69% and 67% respectively) similar to those of the randomized group. The overall rate of live births for women with only two first-trimester miscarriages treated with either heparin or prednisone was 92% (11/12, 95% confidence interval 62% to 100%). However, this improvement was not statistically significant compared with the overall rate for those with more (11/12 vs 21/33, p = 0.13).

The generalizability of our observations was evaluated by comparing outcome data from all groups with those from randomized women with respect to treatment (Table IV). There was no trend (p = 0.8) to a difference in rates of live births when prednisone-treated women were compared with those treated with heparin across all groups. The rate of live births for all groups of prednisone-treated women was 68% (13/19, 95% confidence interval 43% to 87%). The rate of live births for all groups of heparin-treated women was 73% (19/26, 95% confidence interval 52% to 88%). This generalized comparison of live-birth rates had the power (alpha = 0.05, beta = 0.80) to exclude a difference of ≥35% between all women given one of these treatments with respect to live births. Live-birth rates with either treatment were therefore 70% ± 25%. However, fetal deaths after 12 postmenstrual weeks tended to occur more frequently among prednisone-treated women (p = 0.15). The increased risk for preterm delivery, in association with premature rupture of the membranes and preeclampsia, observed among women randomly assigned to treatment with prednisone could be generalized with a high degree of confidence to prednisone-treated groups of women who may have been at different prior risk for fetal death.

Comment

We compared pregnancy outcomes for groups of women with antiphospholipid antibodies and repeated pregnancy losses treated with 80 mg aspirin daily and either standard therapy (40 mg prednisone daily) or adjusted low-dose heparin. Interim analysis of the data did not demonstrate a statistically significant difference between these treatments with respect to the likelihood of a live birth or fetal death. The similarity between prednisone- and heparin-treated women in this respect could be generalized to all such women prospectively ascertained and treated by one of the investigators during this study. Using data from all treated women, we could exclude a 35% difference in live-birth rates between women treated with heparin and those receiving prednisone. For both heparin- and prednisone-treated women the live-birth rate was therefore 70% ± 25%.

However, significantly higher rates of preterm delivery, associated with premature rupture of the membranes and serious maternal morbidity (usually preeclampsia), were observed for randomized women treated with prednisone. These could also be generalized to the other groups of women. Premature rupture of the membranes in prednisone-treated patients may be due to glucocorticoid effects on collagen or may reflect an increased prevalence of subclinical amnionitis. Lubbe and Liggins[22] also noted that premature rupture of the membranes occurred in all pregnancies

Table III. Comparison of women agreeing to randomized assignment with those refusing or ineligible for randomization

Characteristic	*Randomized (n = 20)*	*Refused (n = 13)*	*p Value (R/R)*	*Ineligible (n = 12)*	*p Value (I/R)*
Mean No. of abortions	2.8	2.9	0.7	3.2	0.5
≥1 Loss after 12 wk	9/20	5/13	1.0	9/12	0.15
≥3 Abortions before 12 wk	6/20	4/13	1.0	0/12	0.06
2 Abortions before 12 wk	5/20	4/13	1.0	3/12	1.0
Uncomplicated live birth within 5 yr	0/20	3/13	0.05	2/12	0.1
Lupus anticoagulant present	7/20	1/13	0.1	4/8*	0.6
IgM anticardiolipin antibody only	5/20	5/12	0.4	2/12	0.7
≥3 Criteria for systemic lupus erythematosus	0/20	2/13	0.4	4/12	0.01
Live births in treated pregnancy	15/20	9/13	1.0	8/12	0.7
Live births in treated pregnancy excluding those with just 2 abortions before 12 wk	10/15	6/9	1.0	5/9	0.7
Fetal death after 12 wk in treated pregnancy:	3/20	1/13	1.0	1/12	1.0

p (R/R) value, From two-tailed Fisher's exact test comparing refused group with randomized group. *p (I/R) value,* From two-tailed Fisher's exact test comparing ineligible group with randomized group.

*Results from women currently treated with prednisone for other indications could not be interpreted.

Table IV. Comparison of frequency of live births, fetal deaths after 12 weeks, obstetric complications, and maternal morbidity among women randomly assigned to prednisone or heparin with frequency among treated women refusing or ineligible for randomized treatment assignment

	Randomized		*Refused*		*Ineligible*		*All*		
Prednisone	*Prednisone (n = 8)*	*Heparin (n = 12)*	*Prednisone (n = 3)*	*Heparin (n = 10)*	*Prednisone (n = 8)*	*Heparin (n = 4)*	*Prednisone (n = 19)*	*Heparin (n = 26)*	*p Value*
Live births	6/8	9/12	2/3	7/10	5/8	3/4	13/19	19/26	0.8
Fetal deaths after 12 wk	2/8	1/12	1/3	0/10	1/8	0/4	4/19	1/26	0.15
Preterm delivery	6/6	2/8*	2/2	0/6*	2/5	0/3	10/13	2/17*	0.0002
Premature rupture of membranes	3/6	0/9	0/2	0/6	3/5†	0/3	6/13	0/19	0.006
Serious maternal morbidity (pre-eclampsia + case of cataracts)	4/8	0/10	2/3	0/6	1/6	1/3	7/17	1/19	0.01

Frequency of preterm delivery and premature rupture of membranes was calculated for live births; frequency of maternal morbidity was calculated for all women with pregnancies continuing after 12 postmenstrual weeks. *p* Value (two tailed) from χ^2 test for trend across groups for prednisone compared with heparin treatment.

*Two pregnancies that were electively delivered preterm because of placenta previa and discordant twins were excluded from calculations.

†One woman with premature rupture of membranes at 36 weeks was delivered at term.

of corticosteroid-treated women, unless they were delivered preterm for another indication. Preterm delivery in a study of renal transplant patients (generally treated with doses of prednisone in the range of 0.2 mg/kg/day) was partially attributed to abnormal renal function, but the incidence of premature rupture of the membranes was also higher than expected.[29] In contrast to these observations, no serious morbidity was observed among groups of heparin-treated women. The absence of heparin-associated thrombocytopenia or osteoporotic fractures in heparin-treated women could be due simply to the low frequency of these complications or it could reflect the use of low doses together with prophylactic low-dose aspirin (thrombocytopenia) and supplemental calcium and vitamin D (osteopenia).

From our interim data analysis further pursuit of a possible significant difference in live-birth rates would require that at least 100 women be randomly assigned to either heparin or prednisone treatment, because any difference in this respect between groups was probably ≤25%. Continued evaluation of groups of randomly

treated women with live-birth rates in the range of 70% for a difference of this size did not appear to be justified because of the statistically significant and clinically important risks associated in this study with prednisone treatment. Analysis of the data collected suggested to us that low-dose heparin would be the best choice when treatment is indicated for high-risk pregnant women with antiphospholipid antibodies. Lockshin et al.[25] also concluded that prednisone (usually given in lower doses) did not improve live-birth rates in their series of cases. We did not compare these treatments with aspirin or placebo because the group of women studied were those considered to be at high risk for both fetal loss and puerperal thrombosis. Furthermore, it would be difficult to blind randomly assigned women to the physical effects associated with corticosteroid treatment. Future trials might compare the efficacy of heparin or aspirin with placebo or usual care of women whose medical or obstetric history suggests that they are at low risk for these serious complications. Those at low risk may include women with just two miscarriages before 12 weeks and no history of thrombosis. The live-birth rate for 12 such women treated with either heparin or prednisone was 92% (95% confidence limits 62% to 100%). Other pregnancy outcome parameters such as the frequency of preeclampsia, preterm delivery, and birth weight could be used as surrogate measures of treatment efficacy.

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Necrotizing fasciitis of the vulva

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OBJECTIVE: We attempted to characterize the natural history of necrotizing fasciitis of the vulva.
STUDY DESIGN: The records of 29 nonpregnant women with necrotizing fasciitis of the vulva were evaluated.
RESULTS: These women experienced a rapidly progressing polymicrobial infection. Initially, the infections in many women were thought to be labial cellulitis, appearing mild and innocuous. Delays in recognition and aggressive surgical management were associated with increased morbidity and mortality. Of 15 women with a delay >48 hours between presentation and treatment, 11 died. Twenty of 29 (69%) were diabetic, accounting for 11 of the 14 deaths.
CONCLUSION: Early diagnosis and aggressive surgical debridement in spite of mild symptoms will improve outcome in this serious disease process. (Am J Obstet Gynecol 1992;166:1324-7.)

Key words: Necrotizing fasciitis, diabetes, surgical debridement

Necrotizing fasciitis of the vulva is a surgical emergency.[1-10] This disease may present as an innocuous-appearing labial pustule, and this superficial appearance may mask the underlying pathologic condition. The infection is polymicrobial and treatment is wide excision of infected tissue.[1-10] Since the first descriptions of the disease 100 years ago, authors have called attention to the need for aggressive surgical therapy.[1-10] This report describes our clinical experience over an 8-year period.

Material and methods

We reviewed the medical records of female patients with cellulitis, necrotizing fasciitis, and soft tissue infections of the vulva or the lower abdomen. Twenty-nine patients were examined at North Carolina Memorial Hospital and at Wake Medical Center, during the period January 1983 to January 1990. Included in this study were patients with evidence of primary infection and necrosis of the soft tissue of the external genitalia. Patients with postoperative wound infections, puerperal infections, or perirectal infections were excluded. Patients with inflammatory bowel disease, malignancy, or previous radiation also were excluded. These exclusions were made to focus on necrotizing fasciitis of vulvar origin, unrelated to other genital disease processes.

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Results

The median age of the 29 patients was 59 years, with a range from 28 to 74 years. Fifteen patients had evidence of hypertension or peripheral vascular disease, 8 had chronic renal insufficiency, and 20 were morbidly obese (>50% above ideal body weight). Twenty-four patients were black and 5 were white. Hospital stay ranged from <24 hours to 166 days. Fourteen of 29 patients (48%) died. Table I summarizes the characteristics and clinical course of these patients. Initial presentations varied considerably from chief complaints of mild perineal pain to coma. Seventeen of 29 presented with temperatures >38° C. Two had hyperthermia with temperatures of 36.8° and 35.6° C, respectively. White blood cell counts were >13,000/mm³ in 18 patients.

Twenty patients were diabetic, and 19 of these had abnormal glucose levels on admission. Sixteen presented with blood glucose levels >250 mg/dl. One woman was referred with an intravenous insulin infusion. One patient presented in diabetic ketoacidosis with a blood glucose level of 1426 mg/dl. Another diabetic patient was hypoglycemic on admission with a blood glucose level of 40 mg/dl.

The time course between the patient's presentation to the health care system and definitive surgical therapy varied widely. In general, the longer the delay in surgical debridement, the greater the morbidity and mortality. We did not quantify this relationship because the treatments at other institutions varied widely, as did time from onset of symptoms to admission. Twelve of the 14 deaths occurred in patients with >48 hours between presentation and definitive surgical therapy at our institution. Of the six patients operated on within 12 hours of presentation, 5 survived. The other patient

Table I. Clinical characteristics of patients with necrotizing fascilitis

Patient No.	*Age (yr)*	*Morbidly obese*	*Diabetes*	*Days from diagnosis to treatment**	*No. of operations for debridement*	*Graft*	*Outcome*
1	25	−	−	0	1	−	A
2	28	+	+	0	2	+	A
3	43	+	−	0	1	−	A
4	46	+	+	0	2	−	D
5	48	+	+	0	2	+	A
6	50	+	+	0	−	−	D
7	73	−	+	0	1	−	A
8	47	+	+	1	1	−	A
9	55	+	+	1	1	−	A
10	58	+	−	1	1	−	A
11	59	+	+	1	1	−	A
12	60	−	−	1	2	+	A
13	63	−	+	1	1	+	A
14	67	−	−	1	1	−	D
15	32	+	+	2	2	+	A
16	50	+	+	2	1	−	A
17	55	+	−	2	1	−	D
18	62	+	+	2	1	−	D
19	67	−	+	2	2	+	D
20	75	−	+	2	1	−	D
21	77	−	−	2	1	−	D
22	48	−	−	3	3	+	A
23	50	+	+	3	1	−	D
24	64	+	+	3	1	−	D
25	77	−	+	3	2	−	D
26	67	+	+	4	4	−	D
27	74	+	−	4	2	+	A
28	62	+	+	6	2	−	D
29	64	+	+	10	1	−	D

Patients are listed in order of days from admission to a hospital until definitive treatment. *A*, Alive at hospital discharge; *D*, Died during hospitalization.

*Completed days from hospital admission (at any hospital) until surgical debridement at our institution.

was in septic shock at initial presentation and she eventually died. Seven patients underwent wide debridement between 12 and 14 hours after presentation to a physician. One of these patients was in septic shock at initial presentation, and she died after transfer to our institution. The other 6 survived. Of 8 women operated on between 24 and 48 hours after initial presentation, 2 survived. Finally, only 2 of the 8 subjects who did not undergo operations until ≥48 hours after presentation survived. Length of hospital stay and complications followed similar trends. Three patients had cardiopulmonary arrests; 2 survived, one with severe neurologic deficit. All 3 of these patients had >48 hours between admission and definitive surgical treatment. Adult respiratory distress syndrome developed in 2 patients. Both were in the group with a 48-hour delay in surgical treatment.

Twenty-eight patients were eventually managed with wide local excision and debridement of affected tissues. Nine patients required a second surgical debridement, and 5 other women required three or more operative procedures. Ten patients had skin grafting procedures performed before discharge. Nineteen patients were admitted to the intensive care unit postoperatively. Sixteen of 29 patients required transfusions, with 11 patients required ≥4 U of packed red blood cells.

All patients received broad-spectrum antibiotic therapy including a penicillin or cephalosporin, an aminoglycoside, and clindamycin or metronidazole. Wound cultures yielded more than one organism in 23 patients (79%). Sixteen women (55%) had three or more organisms cultured (Table II). The most commonly cultured organisms were *Peptostreptococcus* (14 patients), *Bacteroides fragilis* (12 patients), and *Escherichia coli, Enterococcus,* and β-hemolytic streptococci. In one patient culture grew *Clostridium tetani* in addition to *E. coli* and *B. fragilis*. She was included in the analysis because *C. tetani*, unlike *Clostridium perfringens*, does not produce its abnormality through myonecrosis.

Comment

Necrotizing fasciitis was described by Joseph Jones, a Confederate Army surgeon, in 1871 as a clinical process wherein, "I have seen the skin in the affected spot

Table II. Organisms cultured from women with necrotizing fasciitis

Organism	*No. of patients**
Peptostreptococcus	14
Bacteroides fragilis	12
Escherichia coli	8
Enterococcus	7
Streptococcus (β-hemolytic)	8
Corynebacteria	3
Diphtheroids	3
Staphylococcus aureus	1
Klebsiella	1
Proteus mirabilis	1
Clostridium tetani	1
Fusobacterium nucleatum	1

*Twenty-three of 29 women had more than one organism cultured. Sixteen women had three or more organisms cultured.

melt away . . . in ever increasing circles."[11] The modern definition of necrotizing fasciitis is a severe infection of the soft tissue in which the major distinguishing characteristic is the presence of extensive necrosis and undermining of the skin, with involvement of tissues up to and occasionally including the deep fascia.[1-10] Our experience suggests that severe pelvic cellulitis and necrotizing fasciitis represent a continuum of clinical conditions requiring aggressive surgical therapy. A definitive diagnosis of necrotizing fasciitis is dependent on the histologic verification of leukocytic infiltration of the fascia, with thrombosis and focal necrosis.[10, 12] However, the management of this disease does not depend on obtaining histologic diagnosis, and the accuracy of frozen section is unknown.

Necrotizing fasciitis of the vulva and perineum is particularly prevalent among diabetics.[2, 5, 6, 8, 12] Among diabetic women, necrotizing fasciitis is particularly prevalent in the vulva and perineum.[6] Rea and Wyrick[8] presented 44 cases of necrotizing fasciitis, among which 8 patients were diabetic. Five of these 8 died (63%). Roberts and Hester[5] reported six cases of necrotizing infections among diabetic patients, including three deaths (50%). Roberts[6] later described 16 cases of necrotizing fasciitis, including 15 among women with diabetes. There were five deaths. Our series includes 11 deaths among 20 diabetic women. It is believed that the extreme mortality among diabetics with necrotizing fasciitis is attributed to the combination of diminished cell-mediated immunity and poor tissue perfusion as a result of peripheral vascular disease.[2] Peripheral vascular disease also has been noted to be a risk factor for this disease. Ten patients had evidence of vascular disease in this series.[3, 8, 10]

Our series was selected to emphasize the patient who represents with vulvar disease not specifically related to other pathologic conditions. This is not to underemphasize that necrotizing fasciitis may occur in many settings. Pregnant patients may also be affected by this disease, with mortality rates of up to 50%.[13-15]

Survival among patients with necrotizing fasciitis requires both early recognition and an appreciation for the associated morbidity and mortality. Patients found to be in high-risk groups need immediate hospitalization and aggressive surgical and antimicrobial therapy.[1, 7, 10, 12] In the current series the 8 patients who had a delay of ≥48 hours in obtaining surgical treatment had a mortality of 75%. Of the 5 patients whose condition was initially managed with simple incision and drainage, all eventually needed wide debridement and one patient died. The delays in surgical treatment occurred at local emergency rooms, gynecologist's offices, and general medicine services at university hospitals. We believe delays are due to the failure of physicians to recognize the severity of this disease process. With a simple incision and drainage procedure, clinicians may not appreciate the underlying inflammation and necrosis. Adequate anesthesia is an absolute necessity for the clinician to obtain debridement that is sufficiently wide.

The earliest signs of the infection are local induration, edema, and marked tenderness.[2, 7, 10, 12] On occasion, a purulent discharge is noted from the affected tissues. It is paramount that clinicians appreciate that the degree of tissue necrosis cannot be predicted by the cutaneous signs. Two patients in our series presented with pustules with "slight" induration, and the condition was managed with hot compresses. Both patients eventually died. It is important that the absence of visible purulent material does not exclude the diagnosis of necrotizing fasciitis. In the course of necrotizing fasciitis, the superficial skin changes may be minimal, even though deeper tissue necrosis is under way. Late signs include skin discoloration and local analgesia, which appear after the infection has spread.[2, 7, 10, 12] At this point the blood supply and innervation of the overlying tissues are interrupted because of thrombosis of vessels and resultant ischemia. Serum calcium levels may be low because of binding with necrosed fat. Crepitus also appears as a late sign because gas-forming anaerobes predominate in the ischemic tissues.[2, 4, 6-9]

Necrotizing fasciitis is a polymicrobial infection. Both aerobic and anaerobic organisms are involved.[2, 4, 6-9, 14] In this series 23 patients were found to have more than one organism and 16 had three or more organisms. This polymicrobial nature of the infection underscores the synergistic activity of the bacteria. Antimicrobial therapy should thus reflect the wide spectrum of bacterial involvement. Antibiotics are an important component in the therapy of necrotizing infections. However, without debridement, antibiotics alone are insufficient therapy. In our series two of three patients whose

condition was initially managed solely with antibiotics died, with no patients' infections resolving with antibiotics alone. Crosthwait[16] reported progression of the infection in 10 of 19 patients treated with antibiotics alone.

The optimal management is wide debridement of all affected tissues. Authors have advocated both blunt undermining of the soft tissue along the fascial plane, until the resistance of viable tissue is encountered,[1, 8-10] and sharp dissection with excision of the full thickness of affected tissue, until bleeding is encountered.[2, 8, 10] Neither method has been shown to be superior, and both techniques were used in our series. The important commonality of both these techniques is that all necrotic tissue is removed.[2, 10] The patient and her family should be prepared for extensive debridement and potentially prolonged hospitalization.

The morbidity and mortality of this disease can be prevented only by a high degree of clinical suspicion. As this series has demonstrated, minimizing the treatment of necrotizing fasciitis does not help the patient.

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The California Maternal Serum α-Fetoprotein Screening Program: The role of ultrasonography in the detection of spina bifida

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Between January 1988 and June 1990, 161 cases of open spina bifida were identified by the California Maternal Serum α-Fetoprotein Screening Program. Eight percent of these cases were not diagnosed by an initial ultrasonographic evaluation. Three defects were not recognized until birth. Ultrasonography is inadequate to identify all cases of open spina bifida. (Am J Obstet Gynecol 1992;166:1328-9.)

Key words: Neural tube defects, α-fetoprotein, ultrasonography

A number of recent articles[1] and letters[2, 3] have suggested an ongoing controversy over the necessity of amniocentesis in the prenatal diagnosis of neural tube defects. Wald et al.[2] summarized the existing studies of spina bifida occurring in women at high risk of having a fetus with a neural tube defect. Thirty-one of 261 (12%) were not detected by ultrasonography. Responding to this report, Nadel et al.[3] contended that only three studies cited by Wald et al. were published after 1986, the year that the ultrasonographic intracranial signs for spina bifida were first reported. In April 1986 offering maternal serum α-fetoprotein (AFP) screening became mandatory in the State of California. This experience with large numbers of pregnant women has allowed new observations of the role of ultrasonography in the detection of neural tube defect. We report our experience with 161 cases of spina bifida ascertained through the California Maternal Serum α-Fetoprotein Screening Program between January 1988 and June 1990.

Material and methods

During the period from January 1988 through June 1990, >640,000 women were screened in the California Maternal Serum α-Fetoprotein Screening Program. Cases with elevated maternal serum AFP values were offered an ultrasonographic examination by an experienced sonographer at one of the 20 prenatal diagnostic centers throughout the State of California. Each center designated as an AFP prenatal diagnosis follow-up center must have at least one sonographer who meets criteria defined by the Genetic Disease Branch. These criteria include board certification, fellowship training in maternal-fetal medicine, or ultrasonography with an emphasis on high-risk obstetric ultrasonographic examinations. In addition, personal experience must include 500 detailed second-trimester examinations specific for the identification of birth defects and a yearly minimum case load. When appropriate, amniocentesis was offered to these patients and AFP testing of the amniotic fluid was performed. If the amniotic fluid AFP level was elevated, an acetylcholinesterase level was ascertained.

Approximately 92% of patients with abnormal maternal serum AFP values had follow-up through one of the program's diagnostic centers. We report our results only from those cases.

Results

Out of the 161 cases of open spina bifida identified, 148 (91.9%) could be said to be detected by ultrasonographic examination. These 148 cases include those diagnosed either with a suspicious finding on the sonogram alone or by ultrasonography before amniocentesis. The remaining 13 cases (8.1%) were not identified by initial ultrasonographic examination. Ten cases were eventually recognized with ultrasonography but only after an amniocentesis yielded an elevated amniotic fluid AFP level and a positive acetylcholinesterase test result. The other three cases were not diagnosed until birth. In two of these instances ultrasonography failed to detect any anomalies and the patients declined amniocentesis. In the third case ultrasonographic measurements of the fetus resulted in a redating of the

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pregnancy. Recalculation of the maternal serum AFP on the basis of this finding placed the value in the negative range and amniocentesis was not performed. Two additional cases of closed spina bifida (not included in the 161 cases) were also missed on ultrasonographic examination. These patients had maternal serum AFP values of 0.57 and 0.62 multiple of the median and were referred for follow-up because of a low maternal serum AFP value. The amniotic fluid AFP values for both were within the normal range, and ultrasonography failed to detect any anomaly.

Comment

The 13 cases of open spina bifida missed on initial ultrasonographic examination occurred in nine of the prenatal diagnostic centers, with one center reporting three of the cases. The data presented in this report are post 1986, the year intracranial signs were first reported. Further, the California AFP program has specific criteria for prenatal diagnostic centers performing AFP follow-up. These include the use of sonographers with extensive training in prenatal diagnosis and second-trimester examinations. However, even in these centers, it is possible that some sonographers have less experience than one of their colleagues and thus are even more likely to miss a defect.

The ultrasonographic detection rate of 91.9% for open spina bifida is significantly enough below 100% to warrant the offering of amniocentesis for patients with elevated maternal serum AFP results. In regard to decision making at moderate maternal serum AFP elevations, four of our 13 missed cases of open spina bifida had multiple of the median values below 3.0. On the basis of our data, we caution against the recommendation to forego amniocentesis with elevated maternal serum AFP results. We agree with Nadel et al.[3] that patients deserve to make an informed decision about whether to proceed with amniocentesis, as long as these choices are based on all available information. It seems reasonable that reassigning risks of neural tube defects on the basis of maternal serum AFP values, the subsequent ultrasonographic findings, and the individual's personal experience before amniocentesis may be indicated in the future.

In an attempt to explain why so many experienced centers missed overt lesions on ultrasonography, a number of factors, including obesity and previous abdominal surgery, may interfere with adequate diagnosis. Transvaginal imaging may decrease image degradation caused by obesity in some patients with high maternal serum AFP values and thus enhance diagnosis, particularly if the head of the fetus is near the cervix. However, transvaginal imaging has a limited field of view and limited maneuverability, both of which decrease its applicability.

The knowledge that amniocentesis will be offered to women whose scans do not reveal an obvious abnormality may influence the detail of those scans. However, not all patients elect to have an amniocentesis, and thus some cases go undetected.

In summary, our experience with the detection of neural tube defects suggests that ultrasonography remains inadequate as a single entity to identify all cases of open spina bifida.

We acknowledge the following California Maternal Serum α-Fetoprotein Screening Program prenatal diagnosis centers that contributed cases during the study period: California Prenatal Diagnostics Institute; Cedars-Sinai Medical Center; Children's Hospital of Los Angeles; Children's Hospital of Northern California; The Genetics Institute; Harbor–University of California, Los Angeles Medical Center; Kaiser-North, Kaiser Permanente Medical Care Program; Kaiser-South, Kaiser Permanente Medical Group; Los Angeles County–University of Southern California Medical Center; Loma Linda University Medical Center; Long Beach Memorial Medical Center; Prenatal Diagnostics, Inc.; Stanford University School of Medicine; University of California, Davis Medical Center; University of California, Irvine; University of California, Los Angeles; University of California, San Diego; University of California, San Francisco; Valley Children's Hospital.

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Fetal humeral length to detect Down syndrome

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OBJECTIVE: Our aim was to evaluate the utility of ultrasonographic humeral length measurements for detection of fetuses with Down syndrome in the midtrimester of gestation.

STUDY DESIGN: Ultrasonographic biometry data obtained before genetic amniocenteses on 43 fetuses with Down syndrome and 204 randomly chosen normal fetuses were analyzed. Regression equations relating biparietal diameter to humeral length and femoral length were used to calculate ratios of observed-to-expected length and sensitivity and specificity at various cutoff points.

RESULTS: Humeral length in Down syndrome fetuses was significantly shorter than in normal controls ($p < 0.001$). A ratio of 0.90 for observed/expected humeral length yielded a sensitivity of 28%, a specificity of 91%, and positive predictive values of 1.23% and 0.41% in populations at risk for Down syndrome of 1 in 250 and 1 in 750, respectively. The equivalent ratio for femoral length yielded a sensitivity of 19%, a specificity of 91%, and positive predictive values of 0.87% and 0.28% for baseline risks of 1 in 250 and 1 in 750, respectively.

CONCLUSIONS: The sensitivity of fetal humeral length measurements for Down syndrome detection in our hands was remarkably lower than previously reported. Independence of this parameter from currently used serum screening markers has not been established; therefore implementation in screening programs is not advisable at this point. (Am J Obstet Gynecol 1992;166:1330-4.)

Key words: Humeral length, Down syndrome, ultrasonography

The role of ultrasonographic screening for Down syndrome is still evolving. Nuchal fold thickening,[1] hypoplasia of the middle phalanx of the fifth digit,[2] short femoral length,[3, 4] and cardiac defects[5] have been proposed as ultrasonographic markers for trisomy 21 fetuses. A recent study by FitzSimmons et al.[6] suggested that upper-extremity long bones were more frequently shortened than the lower extremities in postmortem specimens of Down syndrome fetuses. These findings were confirmed in an ultrasonographic study of 24 Down syndrome fetuses under highly standardized scanning conditions.[7] The current study evaluates humeral length measurements obtained from 43 Down syndrome fetuses and compares the screening utility of this parameter to femoral length measurements and a combination of the two.

Material and methods

From Jan. 1, 1985 to Dec. 31, 1990, 132 fetuses were prenatally diagnosed with Down syndrome at the Division of Maternal-Fetal Medicine and the Department of Human Genetics, Yale University. We analyzed ultrasonographic biometry data of 43 Down syndrome fetuses, with a gestational age between 15 and 21 menstrual weeks, who were scanned before amniocentesis for advanced maternal age. Only scans with complete data on the biparietal diameter (BPD), humeral length, and femoral length were included in the study. All scans in which the fetal karyotype was known at the time of the examination were excluded from the study. The ultrasonographic examinations were performed by more than 15 sonographers with varying levels of experience and training.

Ultrasonography equipment used during the study period included General Electric models RT 2600 and 3000, Ultramark 4 and Ultramark 9 (Advanced Technology Laboratories), Acuson model 128, and Aloka models 650 and 680 (Corometrics). Measurements were obtained with linear, sector, and curvilinear transducers, based on sonographer preference. The BPD was obtained in the axial plane, at the level of the thalami, third ventricle, and cavum septum pellucidum. The calipers were placed on the outer and inner borders of the proximal and distal skull plates, respectively. Humeral and femoral length measurements were performed in the longest dimension of the bone.

Our control group consisted of 204 fetuses with normal karyotypes who were scanned at the same gestational age interval during this 6-year period. To control for variations in scanning technique, equipment, and cumulative experience during the study period, each

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Down syndrome fetus was matched with five karyotypically normal fetuses scanned in the same year. Systematic sampling was performed by dividing the total number of genetic amniocenteses for advanced maternal age during each 1-year period by a factor that would provide five control cases per affected case. When data from a selected record were incomplete, the record was deleted without replacement. This resulted in a control group of 204 cases.

Regression analysis was performed to evaluate the relationships between BPD and humeral length, as well as between BPD and femoral length. An expected humeral and femoral length was then calculated from the regression equation for each BPD. A ratio of observed-to-expected humeral length and femoral length was used to determine the specificity, sensitivity, and positive predictive value of the 43 Down syndrome fetuses.

Selective data from this data set will also be evaluated in the future as part of a multicenter study initiated by Dr. Charles Lockwood.

Results

Table I lists individual BPD, femoral length, and humeral length measurements in 43 Down syndrome fetuses. The regression equation relating BPD to humeral length in this population is: Expected humeral length $= -5.4282 + (0.7542 \times \text{BPD})$; $R^2 = 0.77$; $p < 0.001$. Fig. 1 shows a graphic display of the regression line (line a) of humeral length on BPD ± 2 SD, obtained from 204 karyotypically normal fetuses. Line b represents the regression line for the 43 Down syndrome fetuses. Lines a and b are statistically different ($p < 0.001$).

Fig. 2 shows individual values for humeral length in relation to BPD for 43 Down syndrome fetuses plotted on the regression line from the control fetuses with 95th and 5th percentile limits. In spite of the statistically significant difference between regression equations for normal and Down syndrome fetuses (Fig. 1), only one out of 43 measurements was below the 5th percentile. Eight fetuses had humeral length measurements above the expected value and two fetuses had measurements at or above the 95th percentile.

The regression equation for femoral length on BPD in Down syndrome fetuses was: Expected femoral length $= -6.4047 + (0.7849 \times \text{BPD})$; $R^2 = 0.74$; $p < 0.001$.

The regression lines of femoral length on BPD in the normal and Down syndrome groups were statistically different ($p < 0.05$). In 12 cases the humerus was equal in length to the femur; in 22 cases the humerus was shorter and in nine cases longer than the femur.

Table II shows the sensitivity, specificity, and positive predictive value for various measured-to-expected humeral length ratios in women at risk for Down syndrome of 1 in 250 and 1 in 750. A "cutoff" point of 0.90 for measured-to-expected humeral length identifies 12 out of 43 Down syndrome fetuses (28% sensitivity) and has a false-positive rate of 8.8%. The positive predictive value is 1.23% in a population at risk of 1 in 250, which is equivalent to the risk that a fetus of a 35-year-old woman in the mid second trimester would have Down syndrome. In a population at risk for Down syndrome of 1 in 750 (second-trimester risk at age 28), the same cutoff point of 0.90 would yield a positive predictive value of 0.41%.

Respective data on various measured-to-expected femoral length ratios are shown in Table III. Neither sensitivity nor positive predictive value is as good when femoral length is used as compared with humeral length. When humeral and femoral length measurements were combined (ratio ≤0.90 of measured-to-ex-

Table I. Individual BPD, humeral length, and femoral length in 43 Down syndrome fetuses

Patient No.	*BPD (mm)*	*Humeral length (mm)*	*Femur length (mm)*
1	41	29	26
2	44	27	29
3	44	28	28
4	46	28	31
5	44	26	26
6	38	20	19
7	37	23	24
8	36	21	20
9	39	22	23
10	34	19	20
11	36	20	20
12	38	22	25
13	34	18	19
14	39	24	23
15	48	29	31
16	36	23	20
17	36	19	18
18	36	20	20
19	41	20	21
20	30	15	15
21	33	19	19
22	42	26	25
23	40	22	22
24	38	20	21
25	31	19	19
26	41	20	25
27	31	16	17
28	35	25	26
29	48	30	31
30	31	19	21
31	36	20	21
32	33	17	18
33	36	21	21
34	39	24	24
35	35	20	20
36	35	21	20
37	36	21	22
38	37	19	21
39	47	29	31
40	33	17	17
41	47	32	35
42	32	20	21
43	28	15	14

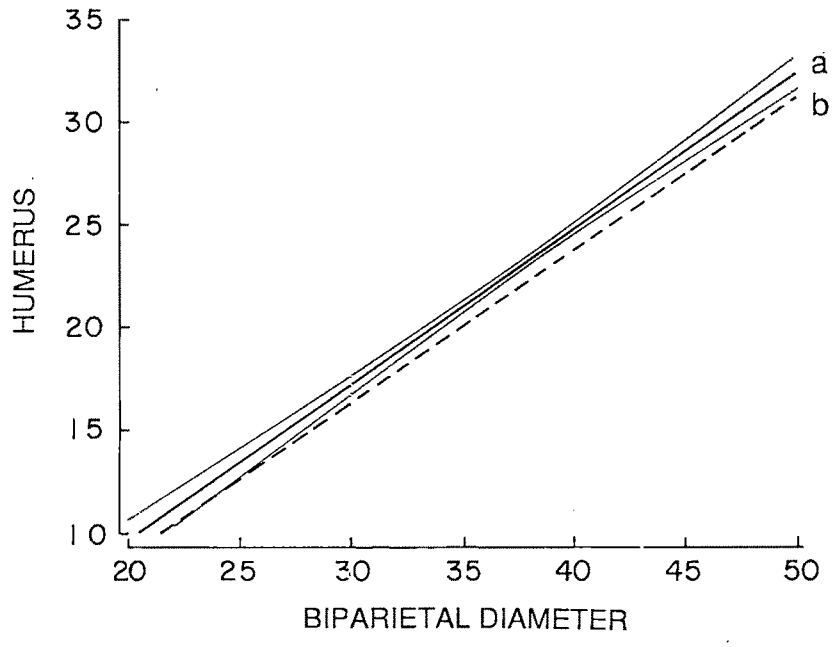


Fig. 1. Regression of humeral length on BPD in karyotypically normal fetuses *(line a)* and ±2 SD *(dotted lines)*. *Line b* represents regression line for Down syndrome fetuses.

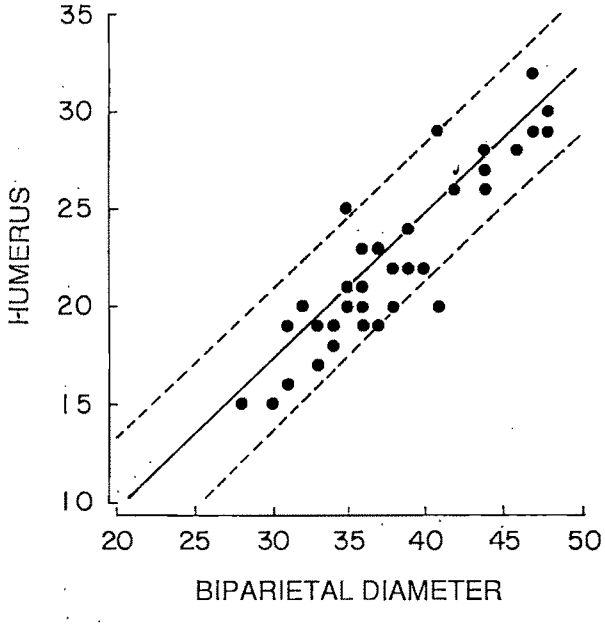


Fig. 2. Regression of humeral length on BPD with 5th and 95th percentiles. Individual values for Down syndrome cases are plotted.

pected length in either humerus or femur), a 30% sensitivity was obtained. However, positive predictive values for baseline risks of 1 in 250 and 1 in 750 were 1.08% and 0.36%, respectively. When both humeral and femoral lengths were combined, whereby a positive finding occurs only when both limbs are short, the specificity was improved but the positive predictive value was lower than for humeral length alone (Table IV).

Comment

Short stature is a well-recognized feature of Down syndrome. Whether shortening of the long bones in the midtrimester fetus is sufficient to aid in the prenatal diagnosis of Down syndrome is controversial. Lockwood et al.[3] and Benacerraf et al.[8] found that the femoral length is significantly shortened in the midtrimester Down syndrome fetus. Lockwood et al.[3] calculated a 50% to 70% sensitivity with a BPD/femur ratio of 1.5 SD above the normal population mean, and Benacerraf et al.[8] reported a 68% sensitivity with a ratio of 0.90 measured/expected femoral length. However, other authors obtained remarkably lower sensitivities.[9] An evaluation of long-bone length in 37 postmortem specimens of Down syndrome fetuses by FitzSimmons et al.[6] found that most measurements fell below the regression lines for normal controls. Furthermore, their data suggested that upper-extremity bones were more frequently shortened than were lower-extremity bones. Benacerraf et al.[7] reported that ultrasonographic measurements of humeral length in 24 midtrimester Down syndrome fetuses were significantly shorter than those in normal controls. An observed/expected humeral length ratio of 0.90 had a 50% sensitivity, a false-positive rate of 6.25%, and a 3.1% positive predictive value in a population at risk for Down syndrome of 1 in 250. Analysis of femoral length in this population of Down syndrome fetuses showed a lower sensitivity (41.6%).

Our data on 43 Down syndrome fetuses confirm that humeral length is significantly shorter in Down syndrome fetuses than in normal controls. Furthermore,

Table II. Sensitivity, specificity, and positive predictive values for different measured/expected humeral length ratios in women at risk for having a Down syndrome fetus of 1 in 250 and 1 in 750

Measured/expected humeral length	*Sensitivity*	*Specificity*	*Positive predictive value*	
			1/250	*1/750*
0.88	0.21	0.93	1.19	0.40
0.90	0.28	0.91	1.23	0.41
0.92	0.30	0.86	0.85	0.28

Table III. Sensitivity, specificity, and positive predictive values for different measured/expected femoral length ratios in women at risk for having a Down syndrome fetus of 1 in 250 and 1 in 750

Measured/expected femoral length	*Sensitivity*	*Specificity*	*Positive predictive value*	
			1/250	*1/750*
0.88	0.12	0.93	0.68	0.23
0.90	0.19	0.91	0.87	0.28
0.92	0.28	0.86	0.80	0.26

Table IV. Sensitivity, specificity, and positive predictive values for different combinations of measured/expected humeral and femoral length ratios in women at risk for having a Down syndrome fetus of 1 in 250 and 1 in 750

Measured/expected bone length		*Sensitivity*	*Specificity*	*Positive predictive value*	
Humerus	*Femur*			*1/250*	*1/750*
0.88	0.88	0.11	0.96	1.04	0.34
0.90	0.90	0.16	0.94	1.02	0.34
0.92	0.92	0.16	0.90	0.67	0.22

a measured-to-expected humeral length ratio of 0.90 identified more Down syndrome fetuses (28%) than did the equivalent ratio for the femur (19%). However, the sensitivity of this ratio in our study was significantly lower than that in the study by Benacerraf et al. This discrepancy could be partially explained by differences between the two ultrasonography laboratories and in study design. Whereas Benacerraf et al. collected cases during a 14-month period, our cases were collected during a 6-year period. It is obvious that ultrasonographic expertise and ultrasonography equipment have changed remarkably in a 6-year period, and less uniformity must be assumed. There were also more sonographers who contributed to this study, as well as a larger variety of ultrasonography equipment and transducers. Differences in long-bone measurements with varying types of ultrasonography transducers have indeed been demonstrated.[10] The higher sensitivity obtained by Benacerraf et al. suggests increased screening efficiency when sonographers and equipment are highly standardized in one laboratory. This also implies that every ultrasonography laboratory should determine its own sensitivity in screening with ultrasonographic measurements. Since our data were collected on a variety of machines, they might be more representative of the inherent variability among various laboratories.

Furthermore, we exclusively evaluated Down syndrome cases for which all three measurements, BPD, humeral length, and femoral length, were available. We assumed that humeral length measurements were not obtained if the measurement was not recorded. It cannot be excluded that humeral length measurements were actually obtained, but discarded, when a sonographer erroneously perceived a disproportionately small humerus to be the result of a measurement error rather than a true growth disturbance.

Since women above the age of 35 are offered amniocentesis for karyotype determination on a routine basis, ultrasonographic humeral length screening would be more applicable to the general population, with an average baseline risk of 1 in 750 for trisomy 21. On the basis of our findings, a positive predictive value of 0.41% could be expected in this population. For every Down syndrome fetus identified in this fashion, 244 amniocenteses would have to be performed on karyotypically normal fetuses. Therefore a measured-to-expected humeral length ratio of 0.90 would

signify a risk for Down syndrome similar to that of maternal age of 35 years, for which routine amniocentesis is currently offered. However, only 5% of the pregnant population are older than 35 when they are delivered.[11] Since 8.8% of our normal controls were below the cutoff ratio of 0.9, implementation of this screening tool would require an additional 8.3% genetic amniocenteses in the general population, with the assumption that fetal humeral length is independent from maternal age. Furthermore, the statistical independence of this screening tool from currently used serum markers for Down syndrome has not been established. Therefore it is conceivable that a substantial portion of fetuses identified by humeral length screening would also be identified by screening with maternal serum α-fetoprotein, human chorionic gonadotropin, and estriol.[12] Such a dependence of parameters would diminish the relative contribution of humeral length screening.

When humeral and femoral length measurements were combined, a cutoff point of 0.90 for observed-to-expected length ratio did not increase the sensitivity obtained for humeral length measurement alone. This finding probably reflects the large degree of concordance in humeral and femoral shortening of Down syndrome fetuses. Small stature in Down syndrome appears to affect all long bones, as suggested by FitzSimmons et al., but is more pronounced in the humerus. Therefore upper- and lower-extremity measurements cannot be used as independent parameters for screening.

Our data support reports by FitzSimmons et al.[6] and Benacerraf et al.[7] of shorter humeral length measurements in midtrimester Down syndrome fetuses, as compared with their normal counterparts. Furthermore, the fetal humerus in Down syndrome appears to be relatively more shortened than the femur. However, the low sensitivity of humeral length screening in our hands and the uncertainty about the statistical independence of this parameter from serum markers for Down syndrome do not support its current use in screening of the general population.

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NORPLANT® SYSTEM
levonorgestrel implants

Lasts 5 years...yet is reversible

Serious as well as minor side effects may occur. The most common side effect which has been observed is menstrual bleeding irregularities.

Please see brief summary of prescribing information on the following page.

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NORPLANT® SYSTEM

levonorgestrel implants

Lasts five years…yet is reversible

BRIEF SUMMARY OF PRESCRIBING INFORMATION. CONSULT THE PACKAGE LITERATURE FOR FULL PRESCRIBING INFORMATION.

Indications and Usage

The NORPLANT SYSTEM is indicated for the prevention of pregnancy and is a long-term (up to 5 years) reversible contraceptive system. The capsules should be removed by the end of the 5th year. New capsules may be inserted at that time if continuing contraceptive protection is desired.

Contraindications

1. Active thrombophlebitis or thromboembolic disorders. 2. Undiagnosed abnormal genital bleeding. 3. Known or suspected pregnancy. 4. Acute liver disease; benign or malignant liver tumors. 5. Known or suspected carcinoma of the breast.

Warnings

A. WARNINGS BASED ON EXPERIENCE WITH THE NORPLANT SYSTEM

1. *Bleeding Irregularities* — Most women can expect some variation in menstrual bleeding patterns. Irregular menstrual bleeding, intermenstrual spotting, prolonged episodes of bleeding and spotting, and amenorrhea may occur, and could mask symptoms of cervical or endometrial cancer. Overall, these irregularities diminish with continued use. Because amenorrhea may occur, missed menstrual periods cannot serve as the only identifier of early pregnancy. Perform pregnancy tests whenever pregnancy is suspected. If pregnancy occurs, the capsules must be removed. Hemoglobin concentrations found in clinical trials generally indicated that reduced menstrual blood loss is associated with NORPLANT SYSTEM use. Blood loss resulting in hemoglobin values consistent with anemia occurred rarely.

2. *Delayed Follicular Atresia* — Atresia of the follicle is sometimes delayed, resulting in enlarged follicles that are clinically indistinguishable from ovarian cysts. In the majority of women, enlarged follicles disappear spontaneously. Rarely, they twist or rupture and surgical intervention may be required.

3. *Ectopic Pregnancies* — Ectopic pregnancies have occurred among NORPLANT SYSTEM users, although clinical studies have shown no increase in the rate of ectopic pregnancies per year among NORPLANT SYSTEM users as compared with users of no method or of IUDs. The incidence among NORPLANT SYSTEM users (1.3 per 1000 woman-years) was significantly below the rate estimated for noncontraceptive users in the U.S. (2.7 to 3.0 per 1000 woman-years). Ectopic pregnancy risk may increase with duration of NORPLANT SYSTEM use and increased weight of the user. Rule out ectopic pregnancy in any patient presenting with lower-abdominal pain.

4. *Breast-feeding* — Steroids are not the contraceptives of first choice for lactating women. Levonorgestrel has been identified in breast milk. Limited data suggests no significant effects on infant growth or health when mothers used the NORPLANT SYSTEM beginning 6 weeks after parturition.

5. *Foreign-body Carcinogenesis* — Rarely, cancers occur at foreign-body intrusion sites or old scars. None has been reported in NORPLANT SYSTEM clinical trials and risk to users is judged to be minimal.

6. *Thromboembolic Disorders* — Remove capsules if active thrombophlebitis or thromboembolic disease develops. With prolonged immobilization removal should be considered.

B. WARNINGS BASED ON EXPERIENCE WITH COMBINATION (PROGESTIN PLUS ESTROGEN) ORAL CONTRACEPTIVES (OCs)

Note: Many of the side effects or risks listed below are thought to be estrogen-related; the association of the NORPLANT SYSTEM progestin-only method to these risks is unknown.

1. *Cigarette Smoking* — Cigarette smoking increases the risk of serious cardiovascular side effects from combined OC use. Risk increases with age and heavy smoking (≥15 cigarettes/day) and is quite marked in women over 35 years old.

2. *Elevated Blood Pressure* — Increase in blood pressure has been reported in combination OC users; prevalence increases with long exposure.

3. *Thromboembolic Disorders and Other Vascular Problems* — An increased risk of thromboembolic and thrombotic disease is associated with combination OC use. Estimate of relative risk is 4- to 11-fold higher for users vs. nonusers.

Cerebrovascular Disorders: Combination OCs increase the relative and attributable risk of cerebrovascular events (thrombotic and hemorrhagic strokes). Generally, risk is greatest among hypertensive women > 35 years of age who smoke.

Myocardial Infarction (MI): An increased risk of MI has been attributed to combined OC use. This is thought to be primarily thrombotic in origin and related to the estrogen component. Increased risk occurs primarily in smokers or women with other underlying risk factors for coronary-artery disease. Relative risk of heart attack for combined OC users is estimated as 2 to 6 times that for nonusers. Absolute risk is very low for women under 30 years old.

Studies indicate a significant trend toward higher MI and stroke rates with increased progestin doses in combination OCs. However, recent data indicated no increased MI risk with past use of levonorgestrel-containing OCs.

4. *Carcinoma* — Recent evidence in the literature suggests no association between OC use and increased risk of breast cancer in the overall population of users. The Cancer and Steroid Hormone (CASH) study also showed no latent effect on breast cancer risk for at least a decade following long-term use. Some of these same studies have shown an increased relative risk of breast cancer in certain subgroups; no consistent pattern has been identified. Some studies suggest an association between combination OCs and an increase in the risk of cervical intra-epithelial neoplasia in some populations of women. The extent to which such findings may be due to differences in sexual behavior and other factors remains controversial. A cause-and-effect relationship between combined OC use and breast or cervical cancer has not been established. Combination OCs may decrease ovarian and endometrial cancer risk. Irregular bleeding patterns associated with NORPLANT SYSTEM use could mask cervical or endometrial cancer symptoms.

5. *Hepatic Tumors* — Hepatic adenomas are associated with combination OC use; estimated incidence is 3 events per 100,000 users per year. Risk increases after 4 or more years of use. Hepatic adenomas are benign but may rupture and cause death through intra-abdominal hemorrhage.

6. *Ocular Lesions* — Retinal thrombosis is associated with OC use and is believed to be related to the estrogen component. However, NORPLANT SYSTEM capsules should be removed if there is unexplained partial or complete vision loss; onset of proptosis or diplopia; papilledema; or retinal vascular lesions. Undertake appropriate diagnostic and therapeutic measures immediately.

7. *Use Before or During Early Pregnancy* — Extensive epidemiological studies reveal no increased risk of birth defects when OCs are used prior to pregnancy. Studies also do not suggest a teratogenic effect when taken inadvertently during early pregnancy. No evidence suggests that risk with NORPLANT SYSTEM use is different.

8. *Gallbladder Disease* — Early studies reported an increased lifetime relative risk of gallbladder surgery in OC or estrogen users. More recent studies, however, indicate that the relative risk of gallbladder disease with OC use may be minimal; this may be related to use of OCs with less estrogen and progestin content.

Precautions

GENERAL:

1. *Physical Examination and Follow-up* — A complete medical history and physical examination should be taken prior to implantation or reimplantation of NORPLANT SYSTEM capsules and at least annually during its use. Exams should include special reference to the implant site, blood pressure, breasts, abdomen and pelvic organs, including cervical cytology and relevant laboratory tests. Rule out malignancy in cases of undiagnosed, persistent or recurrent abnormal vaginal bleeding. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care.

2. *Carbohydrate Metabolism* — Altered glucose tolerance is found in some combination and progestin-only OC users. Effects of NORPLANT SYSTEM on carbohydrate metabolism appear minimal. Observe diabetic and prediabetic patients carefully while using the NORPLANT SYSTEM. Follow women being treated for hyperlipidemias closely if using the NORPLANT SYSTEM. Some progestins may elevate LDL and may render control of hyperlipidemias more difficult. (See **Warnings**.)

3. *Liver Function* — Consider removing capsules if jaundice develops. Steroid hormones may be poorly metabolized in patients with impaired liver function.

4. *Fluid Retention* — Steroid contraceptives may cause some degree of fluid retention. Prescribe with caution, and careful monitoring, in patients with conditions possibly aggravated by fluid retention.

5. *Emotional Disorders* — Consider removing capsules if significant depression occurs since the symptom may be drug-related. Observe carefully those with history of depression and consider removal if depression recurs to a serious degree.

6. *Contact Lenses* — Contact-lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist.

7. *Insertion and Removal* — Insertion is advised during the first 7 days of the cycle or immediately following abortion to insure that the woman is not pregnant and to assure contraceptive effectiveness during first cycle of use. Capsules may be inserted at any time during the cycle provided pregnancy has been excluded and a nonhormonal contraceptive method is used for the remainder of the cycle. Insertion is not recommended before 6 weeks postpartum in breast-feeding women. Follow insertion and removal instructions closely. Healthcare professionals are strongly advised to be instructed in the procedures before they attempt them. Proper insertion just under the skin facilitates removals; proper insertion and removal should result in minimal scarring. If all capsules cannot be removed at first attempt, attempt removal later when the site has healed. Bruising may occur at implant site during insertion or removal. Hyperpigmentation may occur over implant site but is usually reversible following removal. **See Full Prescribing Information for Detailed Insertion/Removal Instructions.**

8. *Infections* — Implant site infection has been uncommon (0.7%); aseptic technique and proper insertion/removal reduces possibility of infection. Institute treatment if infection occurs; remove capsules if infection persists.

9. *Expulsion* — Expulsion of capsules was uncommon; frequency increased when capsule placement was extremely shallow, was too close to incision, or when infection was present. Replace expelled capsule with new sterile capsule. Treat and cure any infection before replacement Contraceptive efficacy may be inadequate with fewer than 6 capsules.

10. *Provisions for Removal* — Advise women that capsules may be removed at any time for any reason. Personnel instructed in removal technique should perform removal on request or at the end of 5 years of usage. Upon removal, dispose of capsules in accordance with Centers for Disease Control Guidelines for biohazardous waste.

DRUG INTERACTIONS: Reduced efficacy (pregnancy) in NORPLANT SYSTEM users has been reported when phenytoin or carbamazepine were used concomitantly. Warn NORPLANT SYSTEM users of possible decreased efficacy with use of related drugs.

DRUG/LABORATORY TEST INTERACTIONS: 1. Sex-hormone-binding globulin concentrations are decreased. 2. Thyroxine concentrations may be slightly decreased and triiodothyronine uptake increased.

CARCINOGENESIS: See **Warnings** section and Full Prescribing Information.

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INFORMATION FOR THE PATIENT: See Patient Labeling. Provide copy of patient labeling to the patient. Advise patients that Prescribing Information is available upon request. Inform prospective users of risks and benefits associated with NORPLANT SYSTEM use, with other forms of contraception, with no contraception, and about insertion/removal procedures. Informed consent from all patients may be desired in light of techniques involved with insertion and removal.

Adverse Reactions

The following have been associated with the NORPLANT SYSTEM during first year of use: many bleeding days or prolonged bleeding (27.6%); spotting (17.1%); amenorrhea (9.4%); irregular (onsets of) bleeding (7.6%); frequent bleeding onsets (7.0%); scanty bleeding (5.2%); pain or itching near implant site - usually transient - (3.7%); infection at implant site (0.7%); removal difficulties affecting subjects - based on 849 removals - (6.2%).

Controlled clinical studies suggest that the following, occurring during the first year, are probably associated with NORPLANT SYSTEM use: headache; nervousness; nausea; dizziness; adnexal enlargement; dermatitis; acne; change of appetite; mastalgia; weight gain; hirsutism, hypertrichosis and scalp-hair loss. The following were reported with a frequency of 5% or greater during the first year and possibly may be related to NORPLANT SYSTEM use: breast discharge; cervicitis; musculoskeletal pain; abdominal discomfort; leukorrhea; vaginitis.

Overdosage

Overdosage may cause fluid retention with its associated effects and uterine bleeding irregularities.

Dosage and Administration

The NORPLANT SYSTEM consists of six Silastic® capsules, each containing 36 mg of the progestin levonorgestrel. The total administered (implanted) dose is 216 mg. Implantation of all six capsules should be performed during the first 7 days of the onset of menses by a healthcare professional instructed in the NORPLANT SYSTEM insertion technique. Insertion is subdermal in the midportion of the upper arm about 8 to 10 cm above the elbow crease. Distribution should be in a fanlike pattern, about 15 degrees apart, for a total of 75 degrees. Proper insertion will facilitate later removal. **(See Full Prescribing Information for Detailed Insertion/Removal Instructions.)**

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Accuracy of frozen-section diagnosis at surgery in clinical stage I and II endometrial carcinoma

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OBJECTIVES: The purpose of our study was to determine if frozen section accurately identifies certain poor prognostic pathologic factors in endometrial carcinoma that are known to be associated with pelvic and paraaortic nodal metastasis, including deep myometrial invasion, poorly differentiated tumor, cervical invasion, adnexal involvement, and poor histologic type.

STUDY DESIGN: The frozen-section pathologic results of 199 patients with clinical stage I and II endometrial cancer were retrospectively compared with permanent-section pathologic findings.

RESULTS: The depth of myometrial invasion (superficial third vs deep two thirds) was accurately determined by frozen-section diagnosis at surgery in 181 of 199 cases (91.0%). The sensitivity of frozen-section diagnosis for deep myometrial invasion was 82.7%, and the specificity was 89.1%. The following tumor characteristics were accurately determined on frozen section at surgery: poorly differentiated tumor (95.0%), cervical invasion (94.0%), adnexal involvement (98.5%), and histologic type (94.0%). Frozen section underestimated deep myometrial invasion in 17.3% of patients with this characteristic and poorly differentiated tumor in 26.3% when compared with permanent-section diagnosis. In patients with unfavorable histologic types, papillary serous and adenosquamous carcinomas were the most commonly misdiagnosed histologic types by frozen section at surgery (70.6%). However, when the preoperative curettage pathologic findings were included, these inaccuracies in tumor grade and histologic type dropped to 15.8% and 35.3%, respectively. Only 13 of 199 patients (6.5%) were not correctly identified by frozen section at surgery as having poor prognostic pathologic features.

CONCLUSION: Frozen section diagnosis at surgery is an important procedure that enables the surgeon to identify patients at high risk for pelvic and paraaortic nodal metastasis. (Am J Obstet Gynecol 1992;166:1335-8.)

Key words: Frozen section, endometrial carcinoma, surgical pathology

In the last four decades endometrial carcinoma has become the nation's most common invasive malignant tumor of the female genital tract. Approximately 33,000 new cases with 5500 deaths are estimated for 1991 in the United States.[1] The combination of total abdominal hysterectomy and bilateral salpingo-oophorectomy is the most common treatment of endometrial carcinoma. Pelvic and paraaortic nodal involvement is associated with certain pathologic findings, including deep myometrial invasion, poorly differentiated tumor, cervical invasion, adnexal involvement, and certain poor histologic types.[2] Postoperative adjuvant radiotherapy is usually recommended for patients with these unfavorable prognostic factors.[3, 4] The factors that affect the prognosis and treatment of endometrial carcinoma, including the depth of myometrial invasion, cervical invasion, adnexal involvement, and retroperitoneal lymph node metastasis, cannot be evaluated clinically before surgery or by fractional curettage. Furthermore, preoperative clinical staging differs from surgical staging in 20% to 50% of cases.[5, 6] Because of the prognostic importance of operative pathologic findings, in 1988 the Oncology Committee of the International Federation of Gynecology and Obstetrics adopted a surgical staging method for endometrial carcinoma. Intraoperative determination of the presence of poor prognostic factors may identify those patients at high risk for pelvic and paraaortic nodal involvement.

We retrospectively reviewed 199 cases of endometrial carcinoma to determine the accuracy of frozen section by comparing the frozen-section diagnosis with the permanent-section diagnosis. The preoperative curettage results were then combined with frozen-section findings to assess their effect on the accuracy of identifying these poor prognostic pathologic features.

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Table I. Correlation of depth of myometrial invasion between frozen-section diagnosis and permanent-section diagnosis

	Permanent-section diagnosis			
Frozen-section diagnosis	*No invasion*	*Inner third*	*Middle third*	*Outer third*
No invasion	23	17	2*	0*
Inner third	2	72	9*	3*
Middle third	0	4*	32	21
Outer third	0	0	10	14

*Clinically important errors of frozen-section diagnosis.

Material and methods

Five hundred forty-one patients with clinical stage I and II endometrial carcinoma were treated at The University of Massachusetts Medical Center (n = 368) and Saint Vincent's Hospital (n = 173), from January 1983 to March 1991. Excluded from analysis were patients who were treated with radiation alone, who were found at surgery to have extrapelvic disease, who did not have frozen sections performed at the time of surgery, or who were referred after hysterectomy; thus 199 cases were available for review. The depth of myometrial invasion, tumor grade, cervical invasion, adnexal involvement, and histologic type were determined by frozen-section diagnosis at surgery. Immediately on its receipt at the frozen-section laboratory, the uterus was opened and the endometrial surface was examined for irregularities of contour and color. Transverse, full-thickness slices were made through the wall of the uterus at 2 to 3 mm intervals for gross inspection. Frozen sections were performed on the areas of deepest tumor extension selected by gross examination. Frozen sections were also performed on the endocervix in patients with a prior positive endocervical curettage or if indicated by gross pathologic findings. The number of frozen sections per uterus varied from one to four according to the gross findings. Care was taken to include both the endometrial surface and the serosal surface in each frozen section. Occasionally, because of the marked thickness of the uterine wall two contiguous sections were required to include both inner and outer surfaces. Frozen and permanent sections were compared with respect to depth of myometrial invasion, tumor grade, cervical invasion, adnexal involvement, and histologic type. Curettage specimens were reviewed for tumor grade and histologic type. Statistical analysis was performed with the Mehta exact test for homogeneity on Egret software (Statistics and Epidemiology Research Corp., Seattle).[7]

Results

Among the 199 study patients, disease was classified as clinical stage I in 164 (82.4%) and as clinical stage II in 35 (17.6%). The patient's ages ranged from 25 to 86 years with a median age of 61.7 years. One hundred three of the patients (51.8%) had one or more of the poor prognostic pathologic features.

The depth of myometrial invasion (superficial third versus deep two thirds) was accurately diagnosed by frozen section at surgery in 181 of 199 patients (91.0%). In 14 patients deep myometrial invasion was missed (middle third invasion, n = 11; deep third invasion, n = 3). In four patients deep myometrial invasion was overdiagnosed (middle-third invasion, n = 4) (Table I). The sensitivity of frozen-section diagnosis for deep myometrial invasion was 82.7% and the specificity was 89.1%. The accuracy was similar for clinical stage I and stage II cases: stage I, 148 of 164 (90.2%); stage II, 33 of 35 (94.3%). When analyzed by tumor grade, the accuracy of the depth of myometrial invasion by frozen-section diagnosis increased as the tumor grade became more poorly differentiated. The depth of invasion was accurately determined in 88.5%, 91.0%, and 95.3% of grades 1, 2, and 3, respectively. However, this difference was not statistically significant (p = 0.258).

The presence or absence of poorly differentiated tumor was correctly determined by frozen section at surgery in 189 of 199 patients (95.0%). Individual tumor grades were accurately diagnosed on frozen section in grade 1, 2, and 3 tumors in 61 of 80 (76.3%), 44 of 73 (60.3%), and 28 of 38 (73.7%) patients, respectively. Poorly differentiated tumor was overdiagnosed in two patients and missed in 10 patients (Table II). When the curettage pathologic results were combined with the frozen-section diagnosis, the frequency of undiagnosed poorly differentiated tumor decreased from 26.3% to 15.8%.

The presence or absence of cervical invasion was correctly diagnosed by frozen section at surgery in 187 of 199 patients (94.0%). Thirty-two patients with clinical stage I disease (19.5%) were found to have cervical invasion (surgical stage II). This was correctly identified on frozen section at surgery in 71.9% of cases. Of 35 patients with clinical stage II, cervical invasion was diagnosed on permanent section in 19. In 16 of these patients (84.2%) the stage of disease was correctly diagnosed by frozen section.

The presence or absence of adnexal involvement was correctly diagnosed by frozen section in 196 of 199

Table II. Correlation of tumor grade between frozen-section diagnosis and permanent-section diagnosis in 199 patients with stage I and II endometrial carcinoma

Frozen-section diagnosis	*Permanent-section diagnosis*			
	No tumor	*Grade 1*	*Grade 2*	*Grade 3*
No tumor	8	14	5	0
1	0	61	22	0
2	0	5	44	10
3	0	0	2	28

patients (98.5%). Twenty-one patients (13.8%) with clinical stage I and II disease had adnexal involvement on permanent section; in 18 (85.7%) of these were detected by frozen section.

Frozen section correctly diagnosed the histologic type in 187 of 199 patients (94.0%). Papillary serous and adenosquamous cell carcinoma were the most common histologic types that were inaccurately diagnosed by frozen section (70.6% of cases). However, when the curettage histologic diagnosis was included, the inaccuracy dropped to 35.3%. Among eight patients with papillary serous carcinoma only three had frozen section at surgery demonstrating a serous histologic type. The addition of curettage histologic results identified 75.0% of cases.

Overall, in 186 of 199 patients (93.5%), the correct diagnosis was made by frozen section at surgery with respect to the status of poor prognostic factors. Thirteen of 103 patients (12.6%) had poor prognostic factors that were not identified at frozen section; 10 had deep myometrial invasion, two had poorly differentiated tumor, and one had a papillary serous histologic type.

Comment

In the current study we compared frozen-section with permanent-section pathologic diagnoses in patients with clinical stage I and II endometrial carcinoma. The goal of frozen section is to identify patients at high risk for pelvic and paraaortic nodal metastasis. Five poor pathologic prognostic factors were evaluated: deep myometrial invasion, poorly differentiated tumor, cervical invasion, adnexal involvement, and certain histologic types. Previous studies have included only patients with clinical stage I disease. However, the endocervical curettage frequently gives false-positive results; therefore patients with clinical stage II disease were included in this analysis.[8]

In clinical stage I and II endometrial carcinoma, frozen section at surgery correctly diagnosed deep myometrial invasion in 91.0%. Malviya et al.[9] reported that frozen section accurately predicted the depth of myometrial invasion in 96.5% of 55 patients with clinical stage I endometrial carcinoma. Similarly, Fanning et al.,[10] studying frozen section in 216 cases of clinical stage I endometrial carcinoma, reported an accuracy for the depth of invasion of 95.0%. Although our data are similar to the previous reports, the accuracy is slightly less. One possible explanation for this may be the fact that multiple pathologists cover the frozen-section service in our hospitals in contrast to a single gynecologic pathology specialist.

In this study the accuracy was slightly greater for clinical stage II and cases with grade 3 curettage histologic results. Creasman et al.[2] reported that as tumor grade becomes less differentiated there is a greater tendency for deep myometrial invasion. These findings are consistent with an increased accuracy of frozen-section diagnosis at surgery in patients with more extensive uterine disease. However, Goff and Rice,[11] studying gross examination for determining the depth of myometrial invasion, reported a decrease in sensitivity as the grade of tumor increased.

Frozen section at surgery correctly diagnosed the presence of poorly differentiated tumor in 95.0% of cases of clinical stage I and II endometrial carcinoma. The previous reports by Malviya et al.[9] and Fanning et al.[10] in clinical stage I endometrial carcinoma were very similar (94.5% and 96.0%, respectively). The separation into three tumor grades by frozen section is less accurate than simply the detection of the presence of poorly differentiated tumor.

Frozen section at surgery accurately diagnosed the presence of cervical invasion in 94.0%. Fanning et al.[10] reported that cervical invasion was accurately diagnosed in 96.0% by intraoperative frozen section in clinical stage I endometrial carcinoma. In contrast, Malviya et al.[9] reported that frozen section at primary surgery predicted cervical invasion in only 66.0% of cases of clinical stage I endometrial carcinoma.

In our study frozen section at surgery accurately diagnosed the presence of adnexal involvement in 98.5%. Fanning et al.[10] reported an accuracy of 98.0% for adnexal involvement detected by intraoperative frozen section in clinical stage I endometrial carcinoma.

At surgery the correct histologic type was diagnosed in 94.0% of cases by frozen section, and papillary serous and adenosquamous carcinomas were the histologic types that were frequently misdiagnosed. Fanning et al.[10] reported that histologic type was accurately diag-

nosed by intraoperative frozen section in 90.0%. They also reported that adenosquamous carcinoma was the most common histologic type inaccurately diagnosed by intraoperative frozen section. However, in the current report the sensitivity for certain unfavorable histologic types increased from 29.4% to 64.7% when curettage pathologic findings were included.

Gross examination of the uterus for determination of deep myometrial invasion instead of frozen-section pathologic analysis has been reported by previous authors.[11, 12] However, the sensitivity of this procedure is only 71.0%, which is significantly less accurate than frozen-section diagnosis at surgery.[12]

Alternatively, other clinical studies may be used preoperatively to determine the presence of one or more of these poor prognostic findings. Gordon et al.[13] and Cacciatore et al.[14] independently reported that the depth of myometrial invasion was correctly predicted by ultrasonography in 79.0% and 80.0% of clinical stage I endometrial carcinomas, respectively. In a report from Obata et al.[15] intrauterine ultrasonography accurately predicted the depth of myometrial invasion in 81.3% of cases of clinical stage I endometrial carcinoma. Gordon et al.,[13] Hericak et al.,[16] and Chen et al.[17] each reported that in clinical stage I endometrial carcinoma magnetic resonance imaging accurately predicted deep myometrial invasion in 71.0%, 82.0%, and 94.0% of cases, respectively. Even though different imaging techniques (ultrasonography, magnetic resonance imaging, and intrauterine ultrasonography) are able to determine preoperatively the depth of myometrial invasion, frozen section at surgery is a more accurate method. Furthermore, these clinical techniques are unable to detect pathologic features such as poorly differentiated tumor, certain unfavorable histologic types, and occult adnexal involvement.

At our institution, after abdominal exploration, cytologic sampling, and palpation and removal of suspicious pelvic and paraaortic lymph nodes, an extrafascial hysterectomy and a bilateral salpingo-oophorectomy are performed. Patients with frozen-section diagnosis of no or superficial myometrial invasion, grade 1 or 2 carcinoma, no cervical invasion, and no adnexal involvement are considered at low risk for nodal metastasis, and lymphadenectomy is not performed unless the curettage had previously demonstrated grade 3 or an unfavorable histologic type. By avoiding lymphadenectomy in such patients, the risk of operative complications, such as hemorrhage, lymphocyst formation, ureteral and vascular injuries, and lengthy operating time were reduced. In contrast, patients with frozen-section diagnosis of deep myometrial invasion, grade 3 carcinoma, cervical invasion, or adnexal involvement are considered at high risk for metastasis to the nodes and selective pelvic and paraaortic lymphadenectomy is performed. Failure to perform lymphadenectomy in patients at high risk for nodal metastasis may result in underdiagnosis of extrauterine disease, subsequently leading to inadequate therapy. This study confirms the accuracy of frozen section, particularly in combination with the curettage findings, in delineating a patient group that is at high risk for lymph node metastasis.

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Hospitalization for pregnancy complications, United States, 1986 and 1987

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OBJECTIVE: The purpose of our analysis was to provide a national overview of the magnitude of the public health burden associated with inpatient care for pregnancy complications.
STUDY DESIGN: We analyzed data from the National Hospital Discharge Survey for 1986 and 1987. We calculated ratios of hospitalizations for pregnancy complications for every 100 hospitalizations involving a birth. Standard errors for these ratios were calculated with RATIOEST, and relative ratios with 95% confidence intervals were calculated for subgroups of interest.
RESULTS: We found that for every 100 hospitalizations involving a birth, there were 22.2 nondelivery hospitalizations for pregnancy complications (14.6 antenatal complications, 7.6 pregnancy loss complications). These ratios were higher for black than for white women (relative ratio 1.4, 95% confidence interval 1.2 to 1.6). The effects of marital status, age, and insurance coverage differed between black and white women, and mean length of stay was longer for black than for white women.
CONCLUSION: Hospitalization for pregnancy complications is far more common than is widely appreciated and is more frequent among black than white women. (AM J OBSTET GYNECOL 1992;166: 1339-44.)

Key words: Maternal morbidity, pregnancy complications, hospitalization, race

Pregnancy is a common condition; in the United States there are now approximately 4 million deliveries,[1] 1.35 million induced abortions,[2] 88 thousand ectopic pregnancies,[3] and, on the basis of recent estimates of early pregnancy loss,[4] likely many more than 1 million spontaneous abortions—adding up to more than 6 million pregnancies per year. Yet there is insufficient information on the epidemiologic characteristics of pregnancy complications or on the magnitude of the public health burden they create. We lack knowledge about which complications of pregnancy cause the most morbidity and about what characteristics of women or their health care are associated with higher risks of complications. This article provides an overview of pregnancy complications involving hospitalization in the United States.

Methods

We analyzed data collected by the National Center for Health Statistics by means of the annual National Hospital Discharge Survey. The design and methods of the National Hospital Discharge Survey have been previously published.[5] The survey uses a sample of nonfederal, short-stay hospitals in the United States, stratified by geographic location and hospital size. From approximately 400 participating hospitals, a sample of approximately 200,000 inpatient records are selected each year and weighted to represent >30 million hospitalizations nationally. From each selected record, information on patient characteristics, medical diagnoses, and surgical procedures is abstracted from the face sheet of the hospital chart. As many as seven discharge diagnoses and four procedures are coded according to the International Classification of Diseases, Ninth Revision, Clinical Modification.[6]

We based our analysis on hospitalizations of women with complications of pregnancy occurring from 1986 through 1987, the most recent years for which data were available. It was necessary to combine 2 years of data to provide a sufficient number of black women for race-specific analyses. Because there were insufficient numbers of women of other races, we restricted race-specific analyses to white and black women. Individual women could have been hospitalized more than once during a pregnancy; it is therefore possible that for some women more than one hospitalization could be represented in our analysis. Therefore this report is based on hospitalization episodes rather than on individual women. The numbers of hospitalizations are rounded to the nearest hundred for presentation.

We selected for analysis hospitalizations in which In-

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Table I. Number* of pregnancy-related hospitalizations, type and year, 1986 and 1987 National Hospital Discharge Survey

Hospitalization type	*1986*	*1987*	*Total*
Antenatal	533,900	583,400	1,117,300
Pregnancy loss	302,500	278,600	581,100
Delivery	3,760,000	3,907,500	7,667,500

*Rounded to nearest hundred.

ternational Classification of Diseases, Ninth Revision, Clinical Modification codes 630.0 through 676.9 (complications of pregnancy, childbirth, and puerperium) or V27.0 through V27.9 (outcomes of delivery) appeared as diagnoses. There were 9,516,000 hospitalizations with these codes in 1986 through 1987.

Our primary interest was hospitalizations during pregnancy that were not associated with induced abortion or delivery. Because hospitalizations that included an induced abortion or delivery could have incidental complications diagnosed that alone would not require hospitalization, including them would lead to overestimating the number of hospitalizations required for pregnancy complications. Therefore we excluded hospitalizations that (1) occurred postpartum (62,400), (2) involved an induced abortion (87,900), and (3) included a delivery (7,667,500). These exclusions left roughly 1,698,000 nondelivery hospitalizations related to complications of pregnancy for our analysis. We used the delivery hospitalizations to calculate a hospitalization ratio (as described later).

We divided the nondelivery hospitalizations into two main categories for analysis: antenatal hospitalizations and hospitalizations related to pregnancy loss. Antenatal hospitalizations were those in which a woman entered the hospital with a complication of pregnancy and was still pregnant when she was discharged. Hospitalizations related to pregnancy loss were those in which a woman experienced a complication of pregnancy and left the hospital no longer pregnant and without delivering a live or stillborn fetus (provided that no induced abortion procedure was performed during the hospital stay).

We used information gathered at the time of admission to classify patients according to whether they had private health insurance. Those whose expected source of hospital payment consisted of any form of private or commercial insurance were considered to have private insurance, whereas those with expected government reimbursement or self-payment were considered not to have private insurance. Those with other designations (<5% of records) were excluded from this classification.

We examined disease burden as a ratio of hospitalizations for pregnancy complications per 100 hospitalizations involving delivery of a stillborn or live infant. Standard errors for ratios were calculated with RATIOEST,[7] a statistical procedure that takes into account the design features of complex multistage surveys such as the National Hospital Discharge Survey. Relative ratios were calculated to compare the ratios of particular subgroups, and 95% confidence intervals for these relative measures were calculated with the Taylor series linearization method.[8] All significantly elevated or reduced relative ratios were directly standardized in a univariate fashion for every other significant factor, to the distribution of that characteristic among all deliveries. The only standardized measures that differed from the nonstandardized did so in a direction further from the null. Therefore for statistical purposes the nonstandardized ratios are reported here; in a few instances these represent a slight underestimate of the true effect.

Mean length of stay and its standard error was calculated for each race with SESUDAAN.[9] We used the *t* test to evaluate differences in mean length of stay between races.

Results

In 1986 and 1987 there were an average of 849,200 pregnancy-related hospitalizations per year that did not involve a delivery, an induced abortion procedure, or a postpartum diagnosis; this yielded an overall ratio of 22.2 hospitalizations for pregnancy complications per 100 delivery hospitalizations, 14.5 antenatal hospitalizations and 7.6 pregnancy loss hospitalizations per 100 deliveries (Table I). Black women had a 40% higher ratio of hospitalizations (both antenatal and pregnancy loss) per 100 deliveries than did white women (Table II). Other demographic risk factors analyzed appeared to show different patterns between black and white women as well. Therefore we stratified all further analyses by race.

Antenatal hospitalization. Table III presents antenatal hospitalization ratios by selected characteristics. The ratios varied with age and race; for white women the ratio decreased with age whereas for black women the trend was in the opposite direction. Among the women for whom marital status was known, 20.5% of white women and 64.3% of black women were unmarried at the time of hospitalization. The antenatal hospitalization ratio was significantly higher among un-

Table II. Hospitalization for pregnancy complications by type and race, 1986 and 1987 National Hospital Discharge Survey

	*No. of hospitalizations**	*No. of deliveries†*	*Ratio‡*	*Relative ratio*	*95% Confidence interval*
Antenatal					
White	682,500	4,907,300	13.9	1.0	Referent
Black	215,300	1,108,600	19.4	1.4	1.2-1.6
Pregnancy loss					
White	368,800	4,907,300	7.5	1.0	Referent
Black	117,900	1,108,600	10.6	1.4	1.2-1.6

*Not shown are data from 219,900 hospitalizations with unknown race and 94,000 of "other" race.
†Not shown are data from 1,136,100 deliveries with unknown race and 515,500 of "other" race.
‡Number of hospitalizations per 100 deliveries.

Table III. Antenatal hospitalization by race, 1986 and 1987 National Hospital Discharge Survey

	White				*Black*			
	No. of hospitalizations	*Ratio*	*Relative ratio*	*95% Confidence interval*	*No. of hospitalizations*	*Ratio*	*Relative ratio*	*95% Confidence interval*
Age*								
15-19 yr	105,800	20.7	1.8	1.4-2.2	42,700	17.7	0.7	0.5-0.9
20-34 yr	530,900	13.3	1.1	0.9-1.4	156,400	19.6	0.8	0.5-1.0
35-44 yr	42,400	11.6	1.0	Referent	12,700	25.4	1.0	Referent
Marital status†								
Married	398,600	12.2	1.0	Referent	63,000	18.9	1.0	Referent
Unmarried	134,900	19.5	1.6	1.3-1.9	113,400	19.1	1.0	0.8-1.3
Private insurance‡								
Yes	418,200	12.5	1.0	Referent	69,900	18.1	1.0	Referent
No	240,500	17.6	1.4	1.1-1.7	136,400	20.6	1.1	0.9-1.4

*Excluded are 2000 hospitalizations of white women and 900 hospitalizations of black women with age unknown and 1400 hospitalizations of white women and 2500 of black women with age <15 or >44 years.
†Excluded are 148,900 hospitalizations of white women and 38,900 hospitalizations of black women with marital status unstated.
‡Excluded are 23,800 hospitalizations of white women and 9000 hospitalizations of black women with unclassifiable insurance status.

married than among married white women. Marital status was not a differentiating factor among black women. Similarly, among the women for whom health insurance status was classifiable, 36.5% of white women and 66.1% of black women were uninsured. The antenatal hospitalization ratio was significantly increased among uninsured white women but not among uninsured black women.

The most prevalent among all listed diagnoses were preterm labor (344,000), genitourinary infection (112,600), early pregnancy hemorrhage (102,000), excessive vomiting during pregnancy (96,600), pregnancy-induced hypertension (76,100), and diabetes mellitus (62,200). Fig. 1 shows the proportion of all antenatal hospitalizations that included these diagnoses. Among these diagnoses, the mean length of stay was longest for diabetes mellitus (4.4 days for whites, 5.5 days for blacks) and shortest for preterm labor (1.8 for whites, 2.4 for blacks). For all antenatal diagnoses combined the mean length of stay for blacks was 3.3 days, significantly longer than the 2.5 days for whites ($p < 0.01$). Overall, antenatal hospitalizations accounted for 2,948,700 hospital days for the period 1986 through 1987.

Pregnancy loss hospitalizations. Table IV presents pregnancy loss hospitalization ratios by selected characteristics. In general, the ratios increased with age for both whites and blacks. For white women the ratio was increased among the unmarried, whereas for black women marital status was not a differentiating factor.

The most prevalent among all listed diagnoses were spontaneous abortion (138,400), ectopic pregnancy (137,700), unspecified abortion (135,100), and missed abortion (51,780). Fig. 2 shows the proportion of all pregnancy loss hospitalizations that included these diagnoses. The mean length of stay was longest for ectopic pregnancy (3.6 days for whites and 4.7 days for blacks). For all pregnancy loss diagnoses combined, the mean length of stay for blacks was 2.7 days, significantly longer than the 1.9 days for whites ($p < 0.01$). Overall, pregnancy loss hospitalizations during the 2-year period accounted for 1,199,500 hospital days.

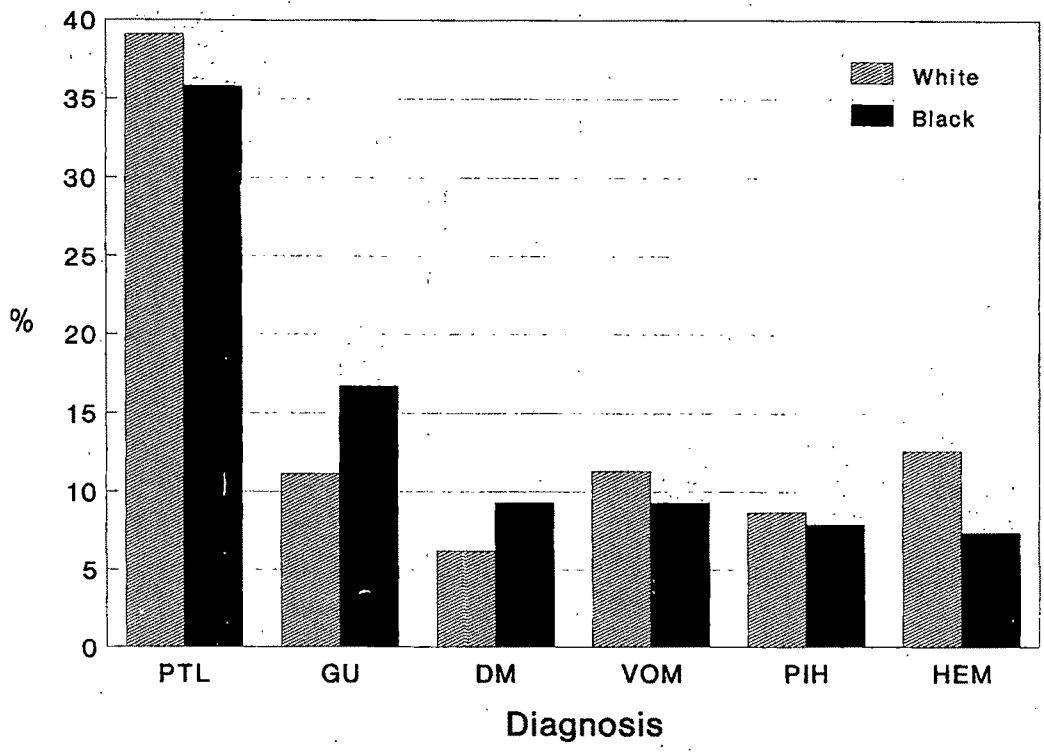


Fig. 1. Percentage of antenatal hospitalizations with leading diagnoses. *PTL*, Preterm labor; *GU*, genitourinary infection; *DM*, diabetes mellitus; *VOM*, vomiting; *PIH*, pregnancy-induced hypertension; *HEM*, early pregnancy hemorrhage. See text for explanation.

Table IV. Pregnancy loss hospitalization by race, 1986 and 1987 National Hospital Discharge Survey

	White				*Black*			
	No. of hospitalizations	*Ratio*	*Relative ratio*	*95% Confidence interval*	*No. of hospitalizations*	*Ratio*	*Relative ratio*	*95% Confidence interval*
Age*								
15-19 yr	37,000	7.2	1.0	Referent	16,600	6.9	1.0	Referent
20-34 yr	282,500	7.1	1.0	0.8-1.2	87,800	11.0	1.6	1.1-2.1
35-44 yr	46,500	12.7	1.8	1.3-2.3	11,200	22.4	3.3	1.9-4.6
Marital status†								
Married	203,700	6.2	1.0	Referent	33,200	9.9	1.0	Referent
Unmarried	83,500	12.1	1.9	1.5-2.3	65,100	11.0	1.1	0.9-1.4
Private insurance‡								
Yes	254,700	7.6	1.0	Referent	49,000	12.7	1.0	Referent
No	103,900	7.6	1.0	0.8-1.2	63,600	9.6	0.8	0.6-0.9

*Excluded are 1600 hospitalizations of white women and 1100 hospitalizations of black women with age unknown and 1200 hospitalizations of white women and 1300 of black women with age <15 or >44 years.

†Excluded are 81,700 hospitalizations of white women and 19,600 hospitalizations of black women with marital status unstated.

‡Excluded are 10,200 hospitalizations of white women and 5300 hospitalizations of black women with unclassifiable insurance status.

Comment

For the period 1986 through 1987 there were approximately 850,000 hospitalizations each year to manage pregnancy complications independent of a delivery, an induced abortion procedure, or a postpartum condition. For every 100 delivery hospitalizations there were over 22 hospitalizations for pregnancy complications. We consider this an underestimate of the actual magnitude of the problem of hospitalization for pregnancy morbidity, because many deliveries occur during hospitalizations involving unsuccessful or extended managment of complications (e.g., preterm labor, pregnancy-induced hypertension). As described above, these hospitalizations were excluded from the numerator in our analysis to avoid counting incidental complications that alone would not have required hospitalization.

Both for hospitalization for antenatal conditions and

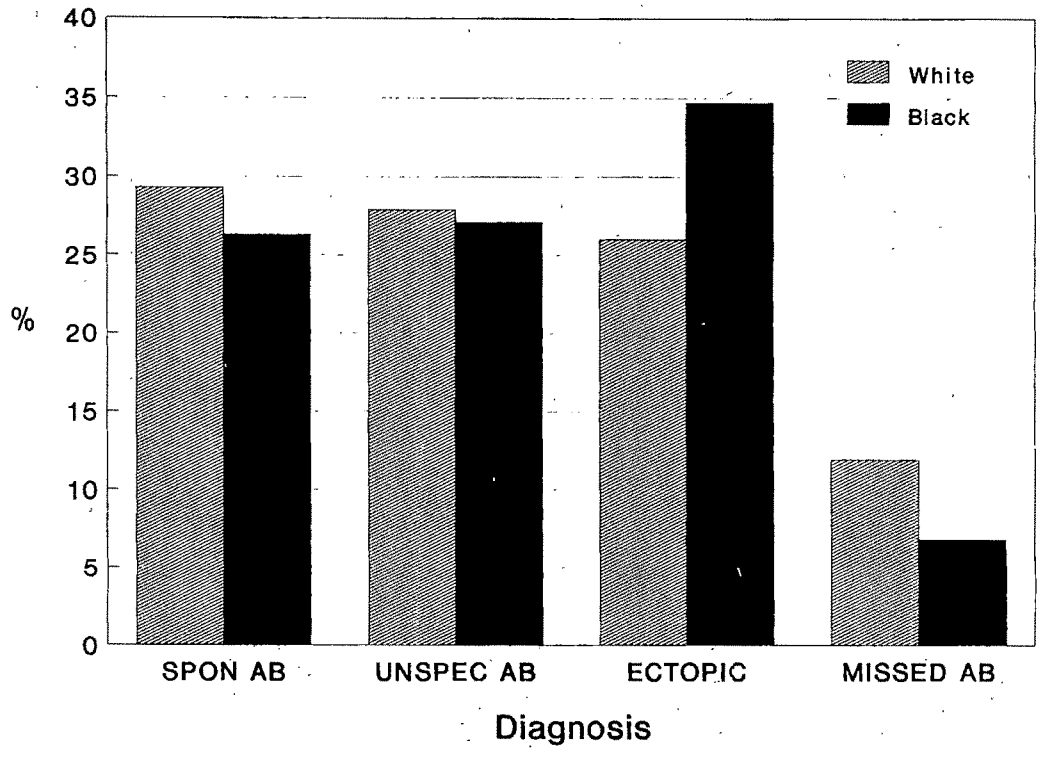


Fig. 2. Percentage of pregnancy loss hospitalizations with leading diagnoses. *SPON AB*, Spontaneous abortion; *UNSPEC AB*, unspecified abortion; *ECTOPIC*, ectopic pregnancy; *MISSED AB*, missed abortion. See text for explanation.

for pregnancy loss management, blacks had a 40% greater ratio of hospitalizations per 100 deliveries than did whites. Potential explanations for this race difference include greater incidence of pregnancy complications among blacks, greater likelihood of inpatient versus outpatient care, greater likelihood of multiple hospitalizations during pregnancy, or greater severity of clinical presentation. The latter two hypotheses are consistent with the fact that black women tend to receive less adequate prenatal care than white women.[10-12] The reasons for this racial discrepancy are subject to much debate and include the possibility of less access to care, negative attitudes toward the medical care system, and other barriers to care.[13, 14] Lacking the availability of a trusted health professional to consult about problems, black women may be more likely to defer care until symptoms become severe enough to demand immediate attention. Moreover, the quality of care and the likelihood of medical intervention received by blacks is known to differ from that received by whites in several areas, including cardiology, renal transplantation, and general internal medicine.[15-21] If less intervention occurs as well for blacks in prenatal care, problems may be more likely to progress to a later stage, requiring hospitalization, perhaps repeatedly.

At the extremes of severity, if black women are seen with more advanced pregnancy complications than white women, it would be expected that a greater proportion of black women would undergo delivery as the only recourse for treatment of advanced stages of conditions such as preterm labor or pregnancy-induced hypertension. If such were the case, our finding of an elevated antenatal hospitalization ratio among black women would represent an underestimate of the actual race difference, because delivery hospitalizations were excluded from the numerator in our analyses for reasons given above.

The fact that being unmarried was associated with higher antenatal and pregnancy loss hospitalization ratios for white women but not black women is not surprising. Among white women pregnancy in the social setting of being unmarried may be associated with substantial social problems that affect both interaction with the health care system and the presence of risk factors for complications. Among black women, marital status may not be a similar indicator of social circumstances, because the majority of births to black women occur among those who are unmarried[22]; thus pregnancy outside of marriage may not serve as a marker of special risk status among black women.

The discrepant findings regarding the association of health insurance with antenatal hospitalization ratios for black and white women may have a similar explanation. Although most white women have private health insurance, the reverse is true for black women. Thus the lack of health insurance may be a marker for

adverse social circumstances among white women, although not providing such a marker of special risk status among black women. It should be noted that the hospitalization ratios for insured and uninsured black women were each higher than the ratio for uninsured white women.

The limitations of using hospital discharge data for epidemiologic studies are well known. Differentiating multiple admissions of the same patient from hospitalizations of different individuals is often impossible because personal identifying information is not available. For this reason we are unable to determine whether the race difference in hospitalization ratios is attributable to a different frequency of multiple hospitalizations for the same women or to different hospitalization rates in pregnancy. However, at least in the case of the race difference in pregnancy loss hospitalizations, multiple admissions are an unlikely explanation; these conditions are almost always one-time events within a single pregnancy.

The paucity of information in hospital discharge data also limits their utility. Information on pregnancy history, income, education, occupation, life stresses, disease severity, adequacy of prenatal care, and nature of attempted outpatient management are of great importance for understanding hospitalization for complications of pregnancy, but these data were not available.

Nonetheless, existing hospital discharge data are useful for quantifying the magnitude of the public health burden of certain types of health problems, for drawing attention to the importance of more detailed research, and for providing some direction for future studies. This analysis provides an overview of the considerable burden of pregnancy morbidity on hospital services. Pregnancy complications required, on average, over two million hospital days of care per year in 1986 and 1987 and cost over one billion dollars per year in hospital charges alone (on the basis of an average hospital cost of $520 per day[23, 24]). Needless to say, even our surprisingly high estimates give only an indirect indication of the burden of hospitalization on women and their families in terms of loss of productive days, family disruption, emotional anguish, and financial strain.

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Buserelin acetate versus expectant management in the treatment of infertility associated with minimal or mild endometriosis: A randomized clinical trial

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OBJECTIVE: We performed a randomized clinical trial to evaluate the efficacy of intranasal 400 μg buserelin three times daily for 6 months versus expectant management in the treatment of infertile women with pelvic endometriosis stage I or II of the revised American Fertility Society classification.

STUDY DESIGN: Seventy-one consecutive patients (mean age 32 years) were studied at the First Department of Obstetrics and Gynecology, University of Milan, and the Department of Obstetrics and Gynecology, Ospedali Riuniti, Bergamo, between February 1988 and June 1989. Thirty-five women were randomly allocated to buserelin treatment and 36 to expectant management. The baseline distribution of subjects for age, disease stage, and reproductive history was similar in the two groups. All patients were followed regularly; median follow-up was 17 months in the buserelin group and 18 months in the women given expectant management. If pregnancy did not occur within 12 months of randomization, cycles were monitored by ultrasonography and hormone measurements, and when abnormalities were detected clomiphene citrate and human chorionic gonadotropin were administered.

RESULTS: A total of 17 pregnancies were observed both in the buserelin-treated patients and in the expectant management group. The 1- and 2-year actuarial overall pregnancy rates were similar in the two groups, 30% and 61% in the former and 37% and 61% in the latter group, respectively. Spontaneous abortion occurred in five of the 17 pregnancies in the women treated with buserelin and in one of the 17 in those managed expectantly; this difference was, however, not statistically significant (χ^2_1 adjusted for disease stage and use of clomiphene citrate and human chorionic gonadotropin treatment = 3.01, $p = 0.08$). No fetal death or stillbirth was observed.

CONCLUSIONS: Our findings suggest that treatment with gonadotropin-releasing hormone agonists is unlikely to have a marked influence on the reproductive outcome of infertile women with minimal or mild endometriosis. (Am J Obstet Gynecol 1992;166:1345-50.)

Key words: Buserelin acetate, infertility, endometriosis

Pelvic endometriosis is a common gynecologic disease. Because it is often found in patients with otherwise unexplained infertility, it has been commonly implicated as a cause of infertility. Surgical ablation of endometriotic lesions is the most widely performed therapy in advanced stages, but in minimal and mild endometriosis the usual therapeutic approach is laser vaporization of the lesion or pharmacologic suppression of ovarian activity. Currently, four basic hormonal regimens are available: danazol, progestins, gestrinone, and gonadotropin-releasing hormone (GnRH) agonists.

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In spite of the large amount of published clinical data, there is still considerable uncertainty regarding the efficacy of these hormonal treatments for increasing conception rates in early endometriosis.[1, 2] Published series were generally small and subjects had different stages of the disease. Furthermore few randomized trials included untreated or placebo controls. In particular few data are available on the role of GnRH agonists to improve reproductive prognosis in infertile women with minimal or mild pelvic endometriosis.

In this paper we report the results of a randomized clinical trial comparing the efficacy of buserelin versus expectant management in the treatment of infertile women with pelvic endometriosis stage I or II of the revised American Fertility Society classification.[3]

Subjects and methods

Eligible patients were women aged ≤38 years who were trying to conceive and had a laparoscopically confirmed diagnosis of minimal or mild endometriosis

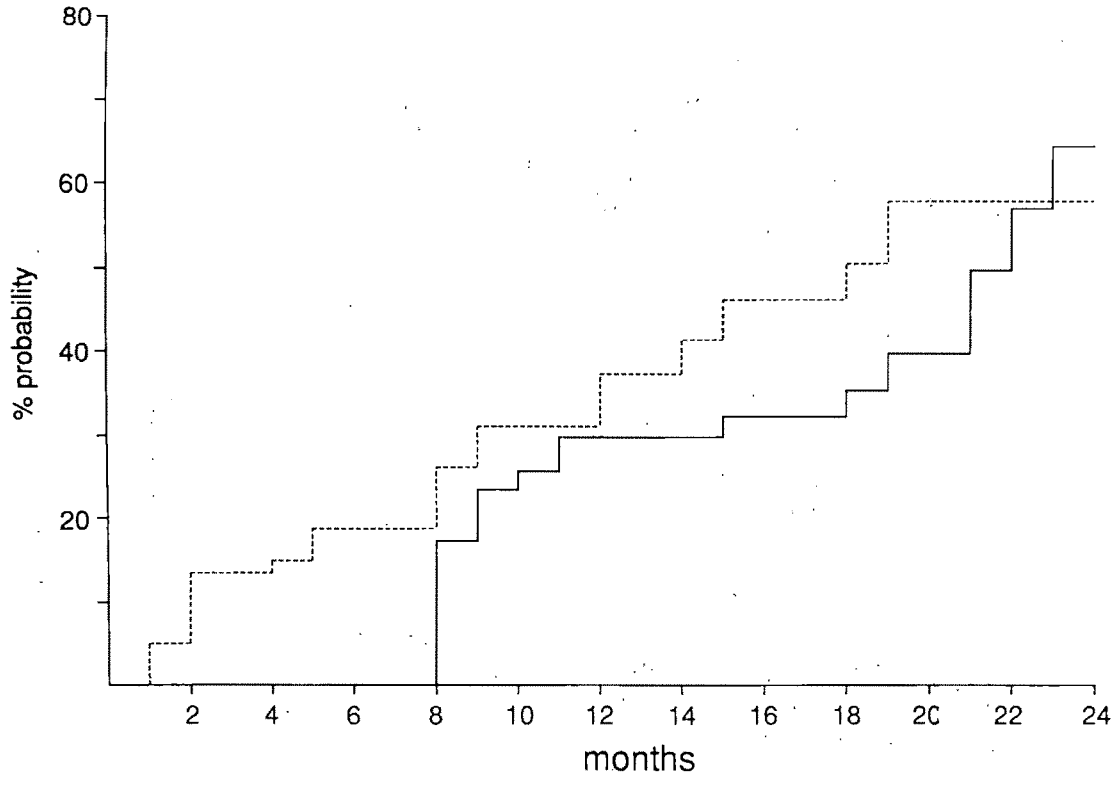


Fig. 1. Overall 24-month probability of becoming pregnant in 71 subjects with minimal or mild endometriosis according to treatment allocation, Milan, Italy, 1988 to 1990. ——, Buserelin-treated group; - - - - -, expectant management group. χ_1^2 adjusted for stage = 0.69, $p = 0.41$ for 12-month analysis; $\chi_1^2 = 0.95$, $p = 0.33$ for 24-month analysis.

(stage I or II of the revised American Fertility Society classification[3]) and otherwise unexplained infertility of ≥2 years. Women were eligible if they had normal results on standard medical and gynecologic examination and hysterosalpingogram, luteal-phase endometrial biopsy, hormone profile (two follicle-stimulating hormone, luteinizing hormone, and estradiol assays in the follicular phase and three progesterone and prolactin assays in the luteal phase), and postcoital test. Further, all partners had two semen analyses to exclude severe dyspermia (>10 × 10^6 sperm per milliliter, quantity >1.5 ml, >30% progressive motility at the third hour, >30% typical forms, absence of agglutination, and leukocyte count <2,000,000 ml). Women with a previous clinical or laparoscopic-laparotomic diagnosis of endometriosis or with any other disease that might affect fertility (for example, uterine malformation or uterine myomas) were specifically excluded from the trial. No women had had previous therapy for endometriosis or infertility. The investigators obtained approval of the protocol from their institutional review board, and each patient gave informed consent. Four eligible women refused to enter the study.

A total of 71 consecutive women observed between February 1988 and June 1989 at the First Department of Obstetrics and Gynecology, University of Milan, and Department of Obstetrics and Gynecology of Ospedali Riuniti, Bergamo, entered the study. The diagnostic laparoscopy was performed under the supervision of L.F. for cases diagnosed in Milan and E.R. for those diagnosed in Bergamo.

Patients were assigned by phone according to a computer-generated randomization list to one of the following treatments: intranasal buserelin (Suprefact, Hoechst, Milan, Italy) 400 μg three times daily for 6 months (35 subjects) and expectant management (36 subjects). If dysmenorrhea or pelvic pain occurred, the patients were asked to use naproxen sodium as first-line treatment.

All patients were examined regularly every 4 months. The median follow-up was 17 months for the patients who received buserelin and 18 months for those who did not. At each follow-up visit a standard gynecologic examination was performed and the occurrence of pregnancy recorded. If pregnancy did not occur within 12 months of randomization, cycles were monitored for

Table I. Distribution of 71 infertile women with pelvic endometriosis according to selected characteristics and treatment allocation, Milan, Italy, 1988 to 1990

	Buserelin		*Expectant management*	
	No.	%	*No.*	%
Age (yr)				
<30	10	29	11	31
30-34	20	57	18	50
>34	5	14	7	19
χ^2_1 trend		0.04 (p = 0.84)		
Disease stage				
I	21	60	21	58
II	14	40	15	42
χ^2_1		0.20 (p = 0.65)		
History of spontaneous abortion				
No	33	94	32	89
Yes	2	6	4	11
χ^2_1		0.66 (p = 0.42)		
Years of infertility				
2	10	29	8	22
≥3	25	71	28	78
χ^2_1		0.37 (p = 0.54)		

two cyles by ultrasonography and hormone measurements. In 29 women the following abnormalities, according to Eissa et al.,[4] were detected: luteinized unruptured follicle cycle (seven cases), luteal phase failure (eight cases), and asynchronous follicle cycle (14 cases). In all these cases clomiphene citrate, 100 mg/day, was given from day 3 to 7 of the cycle, and human chorionic gonadotropin (hCG), 10,000 IU, was administered intramuscularly on the basis of ultrasonographic findings. Induction of ovulation was performed until six cycles.

The present analysis is based on information obtained until June 1990. The outcome of pregnancy with the last menstrual period before June 1990 was checked and data on deliveries collected until February 1991.

Data analysis. We calculated with the actuarial method the cumulative proportion of women who became pregnant, and the curves obtained were compared by the log-rank test.[5] The event data used in the computation of the probability of becoming pregnant were the date of laparoscopy (i.e., of randomization) and the date of the last menstrual period or last follow-up visit.

Tests of statistical significance for the differences in baseline characteristics of the patients and in the frequency of spontaneous abortions on the total of observed pregnancies in relation to treatment allocation were based on the usual χ^2 value, comparing observed and expected numbers of events with the outcome of pregnancy. To allow for the potential confounding effect of stage and ovulation-induction treatment on the outcome of pregnancy, Mantel-Haenszel procedure was used.[6] When indicated, the significance in the linear trend was assessed with the Mantel test.[7]

Results

Table I shows the distribution of the patients according to age, disease stage, history of spontaneous abortion, years of infertility, and treatment allocation. The distribution for these variables was similar in both groups. All patients who entered the study actively tried to become pregnant during the study period. All the buserelin-treated patients took the drug regularly. Amenorrhea was observed in 24 subjects after 1 month of treatment and in all cases after 2 months of treatment. A total of 15 patients in the buserelin (43%) and 14 in the no-treatment group (39%) received drugs to stimulate ovulation (see Subjects and methods).

A total of 17 pregnancies was observed both in the buserelin-treated patients and in the expectant management group (Table II). Of the 34 pregnancies, 13 (seven in the buserelin and six in the no-treatment group) occurred after treatment with clomiphene citrate and hCG. The mean number of cycles in which ovulation was induced in women who conceived after the treatment with clomiphene citrate and hCG was 3.8, with no important difference between the buserelin-treated and the expectant management group.

The 12-month (i.e., before clomiphene citrate + hCG therapy) and the 24-month actuarial overall pregnancy rates were, respectively, 30% and 61% in the buserelin group and 37% and 61% in the expectant management group (χ^2_1 adjusted for stage = 0.69, p = 0.41 for 12-month analysis; χ^2_1 = 0.95, p = 0.33 for 24-month analysis; Fig. 1).

Spontaneous abortion occurred in five (29%) of the 17 pregnancies observed in the buserelin-treated group and in one (6%) of the 17 in the expectant management

Table II. Number of patients at risk and number of observed pregnancies according to month of follow-up and treatment allocation, Milan, Italy, 1988 to 1990.

	Month of follow-up											
	Trial entry	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*
Buserelin												
No. of patients at risk (entering interval)	35	35	35	35	35	35	35	35	35	28	25	24
No. of pregnancies observed in interval	—	—	—	—	—	—	—	—	6	2	1	1
Expectant management												
No. of patients at risk (entering interval)	36	36	34	31	31	28	27	26	26	26	25	23
No. of pregnancies observed in interval	—	2	3	—	3	1	1	—	—	1	—	—

group, but this difference was not statistically significant (χ^2_1 adjusted for disease stage and use of clomiphene citrate and hCG treatment = 3.01; $p = 0.08$).

Comment

This study shows that in infertile women with minimal or mild endometriosis the short-term reproductive outcome was not markedly affected by treatment with buserelin.

Before discussing these results, potential biases should be carefully considered. Our study compares the reproductive outcome in women treated with buserelin and untreated controls in a nonblinded way. However, this should not represent a problem with regard to reproductive prognosis. Considering the frequency of adverse effects related to GnRH agonist treatment, it is, in our opinion, impossible to conduct a "true" double-blind trial. As indicated in the data analysis section, we computed reproductive rates with the date of randomization as an event date. Thus when we computed the 1-year pregnancy rate patients randomized to the buserelin group had actually tried to conceive for only 6 months (because they were under treatment for the first 6 months). We chose to present our results in this way because we think that the 6 months of treatment during which the patients could not conceive should be considered in an overall risk-benefit analysis of the effect of the treatment. In any case life-table analysis performed on only the months of active attempts at conceiving (i.e., considering as starting date the date of randomization in the no-treatment group and the date of stopping treatment in the buserelin group) did not show any statistically significant differences in reproductive outcome at 6 months (data not shown). With regard to other sources of bias, all randomized patients were regularly observed, compliance with the study protocol was generally complete, and the two groups were comparable in terms of age, reproductive history, and disease stage. Another potential limitation of this study is the small sample size and hence its low statistical power. However, with this sample size we can exclude, at the usual level of a study power ($\beta = 0.20$), a double reproductive rate in the treated versus the not-treated women.

According to the study protocol, if a patient had not become pregnant 1 year from randomization, she underwent ultrasonography and endocrine monitoring of two cycles. When we detected abnormalities of folliculogenesis or ovulation as in the patterns described by Eissa et al.,[4] the patients were treated with clomiphene citrate and hCG. This situation must be considered carefully in interpreting the 2-year pregnancy rates and in the generalization of the results of the study. In this regard, the observation that ovulation induction improved fertility rates similarly in both the groups suggests that previous treatment with a GnRH agonist does not affect the efficacy of ovulation induction treatment. Of course, this study cannot evaluate the effect of ovulation induction in improving the prognosis of infertile endometriotic women. It is conceivable that endometrosis impairs folliculogenesis, and ovulation induction may overcome this obstacle, increasing the likelihood of a pregnancy.

In our treated group the abortion rate was greater than in the women given expectant management. This difference was not statistically significant, and in the absence of any biologic explanation it should be largely attributable to chance.

In spite of these potential limitations this is, to our knowledge, the first randomized study reported in the literature that compares GnRH agonist treatment of endometriosis-associated infertility with expectant management. The fertility rates obtained after buserelin treatment were comparable to those observed in

Month of follow-up											
12	13	14	15	16	17	18	19	20	21	22	23
23	23	23	23	22	21	19	16	13	12	10	9
—	—	—	1	—	—	1	1	—	2	1	1
22	17	14	13	11	11	11	10	5	4	4	4
2	—	1	1	—	—	1	1	—	—	—	—

the main clinical studies published so far on the use of GnRH agonists in the treatment of endometriosis. In a large randomized multicenter study[8] comparing danazol, 800 mg/day, and intranasal nafarelin, 400 or 800 μg/day, for 6 months in 149 infertile women there were no statistically significant differences among the 12-month pregnancy rates in the three groups. The pregnancy rates were, respectively, 36%, 30%, and 52%. A randomized study comparing danazol, 600 mg/day, and intranasal buserelin, 1200 μg/day, for 6 months in about 60 patients showed 18-month cumulative pregnancy rates of, respectively, 43% and 38%.[9] The 12-month cumulative pregnancy rates in the randomized study of Dmowski et al.[10] comparing subcutaneous or intranasal buserelin with danazol were 44% and 62%, respectively. In an open study performed by Franssen et al.[11] in which buserelin, 900 μg/day, was given for 6 months the cumulative pregnancy rate after 6 months of follow-up was 32% in 22 previously infertile women.

Until now only four randomized clinical trials[12-15] comparing hormonal treatment with expectant management or placebo have had reproductive outcome as one of the end points. None of these studies reported significant differences between the treatment groups, which received danazol, gestrinone, or medroxyprogesterone acetate or were not treated. In the latter group, the cumulative pregnancy rates at 12 to 30 months were 24% and 57%, respectively.

The failure of buserelin treatment, like that of other hormonal treatments, to improve the infertility associated with minimal and mild endometriosis warrants consideration. First, evidence exists that endometriotic lesions regress after treatment with GnRH agonists,[8, 10, 11, 16] but microscopic examination of the apparently cured pelvic areas has demonstrated the persistence of ectopic endometrium[17] and mitotic activity.[18] Further, there is no evidence of the presumed relation between minimal and mild endometriosis and subfertility.[19, 20]

In conclusion, these results and those of previous studies provide evidence that treament with GnRH agonists is unlikely to have a marked influence on the reproductive outcome in infertile women with minimal or mild endometriosis.

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Magnetic cell sorting and the transferrin receptor as potential means of prenatal diagnosis from maternal blood

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OBJECTIVE: We wanted to test whether the recently described method of using the transferrin receptor system for fluorescence-activated cell-sorter enrichment of nucleated red blood cells can be used for prenatal diagnosis from maternal blood.
STUDY DESIGN: Instead of the laborious, expensive fluorescence-activated cell-sorter system, we used the newly described magnetic-activated cell sorter.
RESULTS: An effective enrichment could be achieved with separation of lymphocyte subsets. With the transferrin receptor, however, the enrichment was very inefficient because of the poor specificity of the antibody itself. Even in umbilical cord blood only 25% of nucleated red blood cells were labeled as demonstrated by immunogold silver enhancement of transferrin receptor–labeled cells.
CONCLUSION: In spite of the availability of a fast and effective separation method (magnetic-activated cell sorter) the use of the transferrin receptor antigen alone is not likely to enable a reliable identification of fetal cells in maternal circulation. (Am J Obstet Gynecol 1992;166:1350-5.)

Key words: Transferrin receptor, fluorescence-activated cell sorter, magnetic-activated cell sorter, polymerase chain reaction

The occurrence of fetal nucleated cells within maternal circulation has raised hopes for a noninvasive method of prenatal diagnosis.[1-3] By application of the polymerase chain reaction, a powerful technique for amplification of specific deoxyribonucleic acid sequences from samples as small as one cell, this goal appears to be accessible. Attempts to amplify Y-specific sequences from the deoxyribonucleic acid of pregnant women have shown that the number of fetal cells in maternal circulation is extremely small, ranging within the lowest detection limit even of the polymerase chain reaction.[3] Yet the risk of contamination—a well-known limitation of the polymerase chain reaction technique—is most prevalent at the highest level of sensitivity and hence often leads to false-positive results.[4, 5]

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Table I. Percentage of nucleated erythrocytes

Sample No.	Blood smear	Cytospin after gradient	Cytospin before MACS	Enrichment factor	Cytospin after MACS	
					Positive sample	Negative sample
1	1	8	3	16	16	7
2	11	2	8	—	9	6
3	5	0	2	—	–*	1
4	7	4	1	—	–*	Cells lysed
5	12	13	9	—	9	19
6	1	0	3	11	11	4
7	4	6	0	1.75	7	4
8	2	5	0	6	12	3

Proportion of nucleated erythrocytes in 100 counted nucleated cells on blood smears and cytospins. Factor of enrichment of nucleated erythrocytes of cytospins of positive samples after magnetic-activated cell sorter *(MACS)* as compared with blood smears.

*Not enough cells left for cytospin centrifugation.

Table II. Total cell count

Sample No.	No. of cells after gradient	No. of cells before MACS	No. of cells after MACS	
			Positive sample	Negative sample
1	2×10^6	1.32×10^6	1.56×10^5	8.15×10^5
2	2×10^6	Not done	1×10^5	6.6×10^5
3	2×10^6	1.03×10^6	1.82×10^4	8.4×10^5
4	2×10^6	1.49×10^6	1.53×10^5	1.23×10^6
5	2×10^6	$*9.2 \times 10^5$	1.38×10^5	9.3×10^5
6	2×10^6	$*9.5 \times 10^5$	1.6×10^5	8.45×10^5
7	3×10^6	2.15×10^6	1.45×10^5	1.52×10^6
8	3×10^6	2.57×10^6	3.3×10^5	1.65×10^6

Total cell count at different stages of the separation procedure as evaluated in a Thoma Zeiss counting cell. *MACS,* Magnetic-activated cell sorter.

*Actual cell number is higher because the cells were partially clotted during immunofluorescence staining.

This risk of contamination caused several authors to evaluate methods of enrichment of fetal leukocytes,[6] nucleated erythrocytes,[7] or trophoblast cells[8] before polymerase chain reaction analysis.

Only flow cytometry has been used to separate fetal cells, but even with the use of multiparameter fluorescence-activated cell sorters, including forward scattering, this method is still time-consuming. We therefore have investigated the usefulness of the recently developed magnetic-activated cell sorter (Milteneyi Biotec GmbH, Bergisch-Gladbach, Germany)[9] with respect to its effectiveness in enriching fetal nucleated erythrocytes; this easier and faster technique would allow a much larger volume of blood to be separated in a given time period.[9]

Fetal nucleated erythrocytes are abundant in fetal blood early in gestation, but are unlikely to be present in the blood of normal adults and express the transferrin receptor antigen on the cell surface.[10] Therefore we chose the enrichment of these cells from fetal umbilical cord blood as a suitable system to evaluate the capacity of the magnetic-activated cell sorter procedure. Before the magnetic separation the cells were sequentially labeled with a monoclonal antibody against the transferrin receptor, biotinylated goat antimouse immunoglobulin G, a fluorescent conjugate, and superparamagnetic microbeads.

Material and methods

Separation of mononuclear cells. Three milliliters of ethylenediaminetetraacetic acid–umbilical cord blood was mixed with 3 ml of buffer (0.3% trisodium citrate and 0.5% bovine serum albumin in phosphate-buffered saline solution: 0.137 mol/L sodium chloride, 0.002 mol/L potassium chloride, 0.008 mol/L sodium biphosphate, 0.0015 mol/L potassium dihydrogen phosphate, pH 7.4) layered over 3 ml of Histopaque-1077 (Sigma, München) and spun at 400*g* for 25 minutes at room temperature. The mononuclear cell layer was isolated and washed three times in buffer 1.

Immunofluorescent staining. Cells (2×10^6) were resuspended in 200 μl of buffer 2 (0.01% sodium azide, 1% bovine serum albumin in phosphate-buffered saline solution) and stained with a 1:10 dilution of anti–transferrin receptor (anti-CD71, Becton Dickinson, Heidelberg) at 4° C for 15 minutes. The cells were washed twice and stained in a 1:10 dilution of biotinylated goat antimouse immunoglobulin G (Amersham, Buckinghamshire, United Kingdom) in buffer 2 for 15 minutes at 4° C and again were washed twice.

Table III. Percentage of transferrin receptor–labeled cells in fluorescence microscopy

Sample No.	Transferrin receptor–positive cells after MACS: Positive sample (%)	Transferrin receptor–positive cells after MACS: Negative sample (%)	Enrichment factor
1	10	2.4	4.1
2	11	2.1	5.2
3	8.7	0.8	10.8
4	1.6	1.1	1.4
5	4.5	1.62	2.8
6	9.5	2.4	3.9
7	3*	2*	—
8	1*	0*	—

Number of brightly fluorescing cells in proportion to all cells visible under phase-contrast microscopy. One hundred cells counted. *Enrichment factor,* Transferrin receptor–positive cells in transferrin receptor–positive samples per transferrin receptor–positive cells in transferrin receptor–negative samples. *MACS,* Magnetic-activated cell sorter.

*Actual cell number is higher because cells were partially clotted during immunofluorescent staining.

After resuspension in 200 μl buffer 2, 20 μl of streptavidin–fluorescein isothiocyanate (250 μg/ml original concentration) (Boehringer, Mannheim, Germany) was added followed by an incubation at 4° C for 15 minutes. In the last step 20 μl of magnetic-activated cell sorter biotinylated microbeads (Milteneyi Biotec) that had been diluted 1:100 (vol/vol) in buffer 2 was added, and the suspension was again incubated for 5 minutes at 4° C.

To test the effectiveness of the magnetic-activated cell sorter procedure, mononuclear cells from three peripheral control blood probes were isolated by Ficoll separation and 10^7 cells were labeled with Leu-7 biotin (anti-CD57) (Becton Dickinson, Heidelberg), then with streptavidin–fluorescein isothiocyanate; after incubation of 10 minutes microbeads were added as described above.

Magnetic-activated cell sorting. The cell suspension was passed over a separation column with a magnetizable matrix that was placed into a magnetic field of extreme strength. Unlabeled cells flowed through the matrix (negative sample), and labeled cells stuck to the column and were eluted after being taken away from the magnetic field (positive sample).

Immunogold silver staining. To test the specificity of the transferrin receptor antibody staining we performed immunogold silver staining in two control umbilical cord blood samples and in peripheral blood samples of two pregnant and one nonpregnant women. Mononuclear cells were separated and stained with the anti–transferrin receptor fluorescein isothiocyanate (5 μl/10^6 cells) as described above. After two washes at 4° C in buffer 2, 2×10^6 cells were mixed with 33 μl of Auro Probe One goat antimouse immunoglobulin G (Amersham) that had been diluted 1:40 (vol/vol) in buffer 2 before and incubated at room temperature for 60 minutes. Auro Probe One is goat antimouse immunoglobulin G linked to gold particles. After two washing steps at 4° C in buffer 2, cells were suspended in buffer 2 in a concentration of 2×10^6 cells/ml, and 100 μl samples were used for cytospins. The slides were fixed for 30 seconds in fixation buffer (20 mg sodium phosphate, 100 mg potassium dihydrogen phosphate in 30 ml water, 45 ml acetone, and 25 ml 37% formaldehyde, pH 6.7), washed three times for 3 minutes in aqua bidest, and air dried overnight.

Enhancement. Silver enhancement was performed according to the suggestions of the manufacturer (Amersham). The silver enhancement time was defined to be approximately 30 minutes for this application. Cytospins were air dried overnight and differentially stained with Diff-Quick (Merz and Dade, Düdingen, CH) in order to identify specific cell populations.

Microscopy. Cytocentrifuge cell preparations were performed after the different steps of staining and the magnetic-activated cell sorter procedure. These and the blood smears obtained from whole blood were differentially stained with Diff-Quick (Merz and Dade) and investigated under a light microscope (Zeiss Orthoplan). Fluorescence microscopy was performed on a photomicroscope (Zeiss Orthoplan, Oberkochen, Germany). Cell counts at different stages of the enrichment procedure were performed on a counting cell (Thoma Zeiss, Oberkochen).

Results

The percentages of nucleated erythrocytes, as counted on slides of fixed and differentially stained cells at different stages of the cell enrichment procedure, are summarized in Table I. The total cell counts at these stages are indicated in Table II. Table III shows the ratio of antibody-labeled cells of positive and negative samples after the magnetic-activated cell sorter procedure as evaluated under the fluorescent microscope; the enrichment factor of transferrin receptor–positive cells after the magnetic-activated cell sorter procedure is also indicated.

The total cell counts show that there is no substantial cell loss during the magnetic-activated cell sorter procedure itself but approximately half of the cells are lost during the immunofluorescent staining (Table II).

Before the enrichment procedure the number of nucleated erythrocytes in blood smears of fetal umbilical cord blood varied between 1% and 12% (Table I). In cytospins of positive samples of magnetic-activated cell sorter–enriched cells this percentage increased in four cases by a factor of 16, 11, 6, or 1.75; in one case the

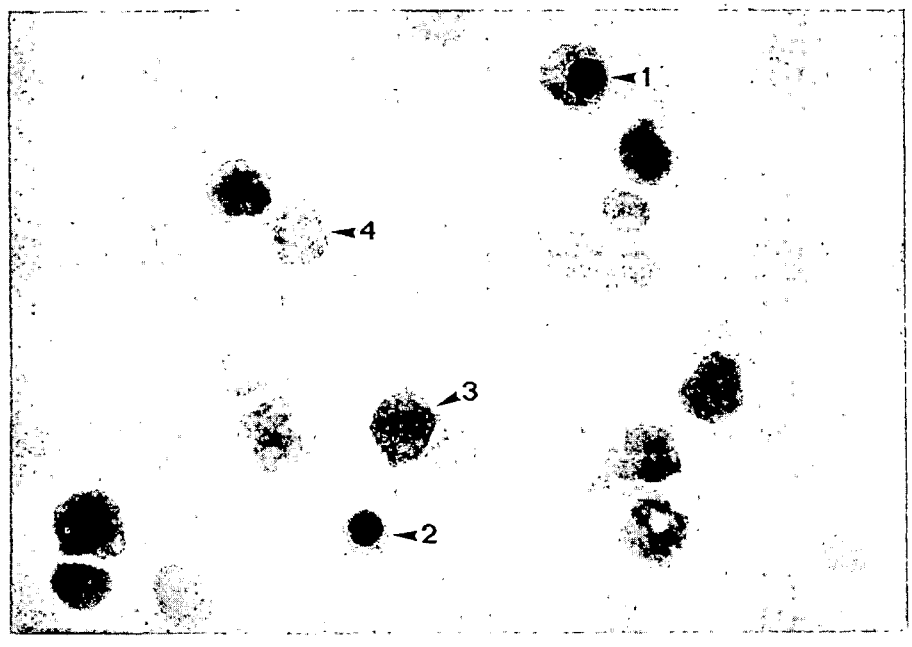


Fig. 1. Diff-Quick–stained cells labeled with anti–transferrin receptor and silver enhanced by immunogold silver staining. *1*, Labeled nucleated erythrocyte; *2*, unlabeled nucleated erythrocyte; *3*, labeled lymphocyte; *4*, labeled nonnucleated erythrocyte.

Table IV. Percentage of Leu-7–labeled cells

Sample No.	*Peripheral blood Leu-7–positive cells (%)*	*Leu-7–positive cells before MACS (%)*	*After MACS*	
			Cell count	*Leu-7–positive cells (%)*
1	47	42	1.7×10^6	96
2	27	23	0.6×10^6	95
3	5	6	2.6×10^5	65

Percentage of Leu-7–positive cells in peripheral blood before and after the magnetic-activated cell sorter *(MACS)* procedure is indicated. The number of Leu-7–positive cells was determined by FACS-SCAN analysis of immunofluorescent-stained cells. The absolute cell number before the separation was 10^7 in all three cases.

percentage of nucleated erythrocytes in cytospins was lower after the separation procedure (Table I). In this case the poor separation correlates with the higher percentage of nucleated red blood cells in the negative control after magnetic-activated cell sorting (Table I), indicating that the magnetic separation was ineffective in this case.

Fluorescence microscopy revealed that two types of morphologically different cells showed specific labeling with the fluorescein marker: (1) small cells expressing a very bright fluorescence and (2) large cells also showing a specific but weaker fluorescence. In positive samples after magnetic-activated cell sorting the proportion of fluorescein-labeled cells was always higher—varying between 1.4 and 10.8 times—than in negative controls. Yet the percentage of transferrin receptor–positive cells evaluated by fluorescence microscopy in positive and negative controls after the magnetic-activated cell sorter protocol did not correlate very well with the actual cell count of nucleated erythrocytes on corresponding cytospins (Tables I and III).

The percentage of Leu-7–positive cells before, during, and after separation with the magnetic-activated cell sorter is summarized in Table IV.

Fig. 1 shows differentially stained cells after immunogold silver labeling of an umbilical cord blood control. The ratios of transferrin receptor–positive versus transferrin receptor–negative cells as evaluated by Diff-Quick are indicated in Figs. 2 and 3. Fig. 2 shows the percentage of transferrin receptor–positive cells of each cell type after differential counts of 400 cells. Only 22% and 17% of the nucleated erythrocytes were labeled in the umbilical cord blood. In the blood of pregnant women only nonnucleated red blood cells were found.

Differential cell counts of each cell type with the corresponding percentages of specifically labeled cells are indicated in Fig. 3. In cord blood the nonnucleated

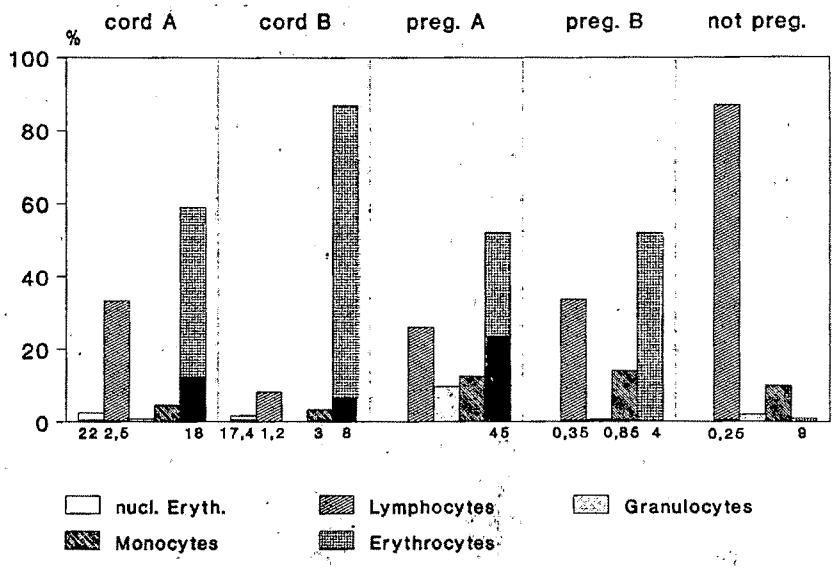


Fig. 2. Differential cell counts of transferrin receptor–labeled and immunogold silver–stained cells from two samples of umbilical cord blood and blood of two pregnant and one nonpregnant women. Proportion of different cell types in 400 cells counted. *Numbers* at bottom of graph indicate proportion of transferrin receptor–positive cells counted within each cell species.

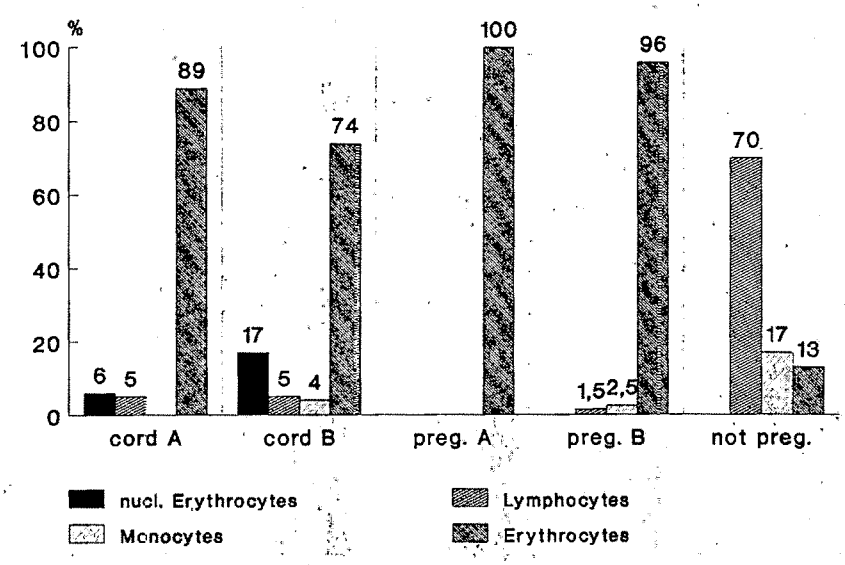


Fig. 3. Differential cell counts of transferrin receptor–labeled and immunogold silver–stained cells. Only transferrin receptor–positive cells were counted. Proportion of transferrin receptor–positive cells of each cell type is indicated at top of each *bar*.

erythrocytes are the prevalent cell type after Ficoll separation, followed by lymphocytes and few monocytes and nucleated erythrocytes; in the nonpregnant female the majority of the separated cells were lymphocytes. The ratio of monocytes, neutrophilic granulocytes, and nonnucleated erythrocytes is <10% (Fig. 3). The pregnant female controls showed somewhat intermediate cell counts after the gradient separation, with nonnucleated erythrocytes in about half of the cells, followed by lymphocytes and comparatively high counts of monocytes and neutrophilic granulocytes (Fig. 3).

Comment

There are two potential types of fetal cells to be separated to identify fetal genes in maternal circulation: trophoblast and nucleated erythrocytes. Because the results regarding the presence of trophoblast material in maternal circulation conflict[8, 11] and because the number of nucleated erythrocytes as a source of fetal de-

oxyribonucleic acid is assumed to be higher. Bianchi et al.[7] used the transferrin receptor antigen, known to be expressed on erythroblasts for enrichment. For enrichment they used the fluorescence-activated cell-sorter method.

Because this method could be too laborious and expensive for application in routine prenatal diagnosis, we evaluated the recently described and faster magnetic-activated cell sorter technique. In control separations of lymphocyte subsets we could show that the enrichment of Leu-7–positive cells was satisfactory in a shorter time period than would be necessary with the fluorescence-activated cell-sorter (Table IV). The magnetic-activated cell sorter has also proved to be well-suited for rare-event sorting.[9] Considering this efficiency of enrichment obtained with magnetic-activated cell sorter the latter can now be considered the most appropriate method in "rare event" situations such as fetal cells in maternal circulation, which probably are in the range of 10^{-6} to 10^{-7} or even lower.[1, 2, 5]

Considering the good rates of enrichment with magnetic-activated cell sorter, it should have been possible to achieve an adequate number of cells for polymerase chain reaction analysis provided there is a good marker for nucleated red blood cells.

Surprisingly, although we optimized conditions such as incubation time or temperature for labeling the nucleated fetal erythrocytes, our results indicate that the specificity and sensitivity of the transferrin receptor antibody is limited: even in umbilical cord blood only <25% of nucleated erythrocytes were transferrin receptor positive. Furthermore, the majority of labeled cells were nonnucleated erythrocytes.

These findings can also serve as an explanation for the observation of Bianchi et al.[7] that after fluorescence-activated cell sorting the number of transferrin receptor–positive cells was 300 to 4000 times higher than the number of fetal cells actually detectable by polymerase chain reaction. Although our experiments have not used the fluorescence-activated cell sorter for enrichment of transferrin receptor–positive cells, the lacking specificity of the transferrin receptor antibody would be expected to preclude an effective isolation by fluorescence-activated cell sorting.

Therefore it seems doubtful whether the use of monoclonal antibodies against transferrin receptor alone is a suitable way to enrich fetal cells in maternal circulation, even if the powerful magnetic-activated cell sorter separation technique is used.

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Training obstetric and family practice residents to give smoking cessation advice during prenatal care

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OBJECTIVE: Our objective was to determine the effectiveness of training obstetric and family practice residents to provide smoking cessation advice.

STUDY DESIGN: The effectiveness of the trained residents' advice was assessed from exit interviews of pregnant smokers taking part in a randomized, controlled trial of smoking cessation advice. Exit interview responses were compared by χ^2 analysis.

RESULTS: Training resulted in significant changes in the advice provided by the residents, with greater emphasis on gaining a commitment to smoking behavior change, but not in the average time providing the advice, approximately 3 minutes. Adherence to the protocol was maintained at 80%. Significantly more women who received the brief structured advice agreed to stop smoking (54%) or cut down their cigarette consumption (28%) compared with women in the control group (14% and 6%, respectively), $p = 0.0001$.

CONCLUSION: The structured advice consistently provided by the trained residents was effective in gaining commitments from pregnant smokers to change their smoking behavior. (AM J OBSTET GYNECOL 1992;166:1356-63.)

Key words: Resident training, smoking cessation advice, prenatal care, pregnancy

The risks of smoking during pregnancy are well established.[1, 2] About 25% of women smokers stop smoking when they become pregnant, and the majority of those who do not stop reduce their consumption of cigarettes by approximately 50%.[3, 4] Several studies of smoking cessation advice delivered during prenatal care have shown significant reductions in smoking in the intervention group compared with the control group,[5-9] whereas others have not.[10] These trials have tested a variety of intervention strategies, including advice from physicians,[10] advice from physicians and a specially designed leaflet,[8] individual advice and counseling,[5] extended advice from public health nurses,[9] brief advice from public health nurses and a self-help manual,[6] and self-help materials sent through the mail.[7] Three of these studies reported a significant increase in the mean birth weight of babies born to mothers in the intervention group compared with those in the control group.[5, 8, 9]

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Recent randomized controled trials of smoking cessation advice delivered by internists[11, 12] or by family physicians[13, 14] have shown the need to identify consistently each patient's smoking status to ensure that appropriate advice is given. The advice provided in these and other studies has included a strong recommendation to quit smoking and an attempt to gain the smoker's commitment to a quit date.[11-16]

Because advice to quit smoking delivered by physicians is cited by smokers as important in their decision to stop smoking[17] and because methods to train physicians to provide smoking cessation advice have shown some success,[13, 14, 18] we examined the effect of training residents in obstetrics and family practice to provide brief smoking cessation advice to pregnant women. This article describes the methods we used to train the residents, the smoking cessation protocol and prompt sheets they were trained to use, the evaluation of the effectiveness of the training in terms of the manner and consistency with which the smoking cessation protocol was followed, and the immediate impact on the women's agreement to change their smoking behavior.

Methods

As part of an ongoing randomized controlled trial of physician-delivered, counselor-supported smoking cessation advice during obstetric care, all obstetric ($n = 19$, 14 female and five male) and family practice ($n = 16$, 11 female and five male) residents at the University of Vermont were trained in a brief smoking cessation protocol between July 1988 and September 1990. The trial

Table I. Demographics, pregnancy status, and smoking history

Characteristic	*Intervention group (n = 125)*	*Basic care group (n = 125)*
Age (yr, mean ± SD)	22.7 ± 5.3	22.7 ± 4.8
Education*		
<High school	55 (44.0)	51 (40.8)
High school	54 (43.2)	59 (47.2)
>High school	16 (12.8)	15 (12.0)
Marital status*		
Married	41 (32.8)	36 (28.8)
Single or other	84 (67.2)	89 (71.2)
Receiving medicaid*	66 (53.7)	70 (56.5)
Primigravid women*	68 (54.4)	69 (56.1)
Length of gestation at first visit (wk, mean ± SD)	14.7 ± 7.3	14.1 ± 6.3
No. of cigarettes/day (mean ± SD)		
Before pregnancy	26.0 ± 10.3	25.6 ± 10.8
At first visit	14.4 ± 7.2	13.3 ± 7.7

There are no significant differences between the two groups.
*These are the number of subjects with this characteristic, with the percentage in parentheses.

is taking place in the Maternal-Infant Care clinic, which provides prenatal care to women with little or no insurance, 40% to 45% of whom are smokers at the onset of pregnancy. Prenatal care is provided by the obstetric and family practice residents during their rotations through the Maternal-Infant Care clinic under the supervision of a faculty member.

Pregnant women who are receiving their prenatal care through the Maternal-Infant Care clinic and who are smoking one cigarette a day or more at their first antepartum visit are randomly assigned either to the intervention group or to the basic care group. Those in the intervention group receive advice from their physician (described subsequently) and immediate referral to a smoking cessation counselor. Those in the basic care group are advised to stop smoking by their physicians and given a booklet on how to do so.

At entry into the study, the demographics, pregnancy status, and smoking histories of the women in the intervention and basic care groups were comparable, as shown in Table I. They tended to be relatively young, 55% were primigravid, most had a high school education or less, more than two thirds were not married, and 55% were receiving Medicaid. On average, they had cut down their smoking from 26 cigarettes per day before pregnancy to 14 per day at their first antepartum visit.

The intervention smoking cessation protocol. The residents are prompted to deliver the smoking cessation intervention by a protocol prompt sheet (Fig. 1) placed in the woman's chart at the first three antepartum visits and again at the fifth and thirty-sixth week visits. The key elements of the intervention delivered by the physicians are as follows: acknowledge the woman's smoking status and any progress with changing her smoking behavior since becoming pregnant, deliver a rationale for stopping smoking and a strong quit message, ask how the woman feels about quitting and acknowledge her response, ask how she could be helped and mention the availability of the smoking cessation counselor in the obstetric practice, elicit a commitment to action and a date for such action (quitting for good, quitting for 24 hours, or cutting down to half her present level of smoking), note the result on the protocol prompt sheet, and refer the woman to the smoking cessation counselor.

The smoking cessation counselor sees the woman immediately after her visit with the physician, counsels her on ways to prepare for her change in smoking behavior, and provides further contact, counseling, support, and follow-up as needed.

This protocol is repeated at the second antepartum visit for those women who are still smoking and again at the third, fifth, and thirty-sixth week visits for those women still interested in stopping smoking. Women who have quit at these visits are praised for their success, encouraged to continue nonsmoking behavior, and referred to the counselor for further support in maintaining their abstinence from cigarettes. For each of these visits the elements of protocol are included as bulleted items on the protocol prompt sheet.

The basic care smoking cessation protocol. Women randomly assigned to the control group receive the basic care advice, which is outlined on a separate protocol prompt sheet. This advice consists of acknowledging the woman's smoking status, delivering a rationale for quitting, giving a strong recommendation to quit, and providing a quit-smoking booklet at the first antepartum visit. These women are not encouraged to set a quit date, nor are they referred to the smoking cessation counselor. No prompts are provided to the residents for this group after the first visit.

Training. Before beginning training, each resident received a description of the training and copies of four

Smoking in Early Pregnancy

Name:_____ SN:_____

Smoking History: Years:_____ # Quits:_____ Longest:_____ Latest:_____

Household: # of other smokers:_____ # of adult non-smokers:_____ # of children:_____

Comments:_____

Date	Weeks	Cig/D	CO	Intent	Progress	MD/ NM	Plan and Date	Seen by Counselor

- Acknowledge smoking status, progress, and CO level. (Non-smokers typically have levels ≤ 8 ppm of CO.)
- Deliver rationale and strong quit message: "I strongly recommend that you quit smoking during your pregnancy."
- "How do you feel about quitting?" (acknowledge her response)
- "How can we help you?" (mention counselor)
- Elicit commitment to action. (quit date, 24 hr quit, cut down to half)
- Note result under "Plan and Date" and refer to counselor.

Date	Weeks	Cig/D	CO	Intent	Progress	MD/ NM	Plan and Date	Seen by Counselor

If she is still smoking

- Acknowledge smoking status, progress, and CO level.
- Deliver rationale and strong quit message.
- "How do you feel about quitting?"
- "How can we help you?"
- Elicit commitment to action. (quit date, 24 hr quit, cut down)
- Note result under "Plan and Date" and refer to counselor

If she is not smoking

- Acknowledge her quitting and give feedback on CO level.
- Praise her progress.
- Emphasize importance of staying quit throughout her pregnancy.
- Refer her to the cessation counselor for help in staying quit.

Fig. 1. Protocol prompt sheet.

Date	Weeks	Cig/D	CO	Intent	Progress	MD/ NM	Plan and Date	Seen by Counselor

If she is still smoking

- "Have you thought about your smoking since the last time you were here?"
- "Do you want to make any changes?"
- If she wants to make a change, refer to the counselor.

If she is not smoking

- Praise her progress.
- Emphasize importance of staying quit throughout her pregnancy.
- Refer her to the cessation counselor for help in staying quit.

Date	Weeks	Cig/D	CO	Intent	Progress	MD/ NM	Plan and Date	Seen by Counselor

If she is still smoking

- Acknowledge her smoking, reinforce any progress.
- "After the child is born, smoking may have an adverse affect on its health."
- Refer to counselor if she requests assistance.

If she is not smoking

- Acknowledge and reinforce her quitting.
- Rationale and support for staying quit after pregnancy.
- "How do you feel about that?"
- "How can we help you?"
- Refer to counselor for assistance.

Fig. 1—Cont'd.

papers concerning smoking cessation advice.[5, 19-21] Each year the residents were trained by a physician and a psychologist in small groups during a 1-hour workshop. This workshop included the following: a review of the project, a description of the advice to be given to subjects in the intervention condition with a rationale for each step, the use of the specially designed protocol prompt sheet to guide this advice, a videotaped demonstration of the advice being delivered by a family physician and by an obstetrician, a role play of the advice by pairs of residents with corrective feedback, and a description of the basic care advice to be given to control group subjects. Residents who were unable to attend a workshop were trained individually.

Refresher sessions and progress. Each resident also participated in a 30-minute individual refresher session

with the psychologist a few days before starting his or her first rotation on the Maternal-Infant Care clinic. During these sessions the intervention and basic care protocols were reviewed, the intervention protocol was role-played with a simulated patient, and appropriate feedback was provided to the resident. Each month the progress of the trial and the residents' adherence to the protocols were discussed by the smoking cessation counselor during Maternal-Infant Care clinic conferences.

Evaluation of resident training. The effectiveness of the training was evaluated in three ways. First, an evaluation of the immediate impact of the training on the content and style of each resident's smoking cessation advice was conducted with the first group of residents trained in 1988. This group consisted of all 12 obstetric residents (three in each postgraduate year), and all eight first- and second-year family practice residents. This evaluation was done by comparing videotapes of the residents providing their usual advice to a simulated patient before training, with videotapes of each resident delivering the intervention protocol to the same simulated patient at the end of their refresher session.

Seven criteria, derived from the intervention protocol and selected before the videotapes were viewed, were used to rate the videotapes. These criteria were as follows: The resident provides a rationale for quitting, provides a clear, unambiguous quit message, acknowledges the woman's feelings about quitting, asks an open-ended question, elicits a commitment to a quit day, encourages the woman to select a day when she will make some change in her smoking, and indicates that the change date will be entered in the chart or that an enquiry will be made about smoking at the next visit. The tapes were scored independently by two trained raters who were blind to the training status of the resident. Scoring involved noting the presence or absence of each of the seven criteria, such that a higher score represented the inclusion of more of the desired components of the intervention protocol. Scores could range from 0 to 7. The overall interrater reliability across all seven criteria was 84%. The time the resident spent delivering the advice was also recorded.

As a second way of evaluating the training, each trained resident's adherence to the protocols was assessed from exit interviews with all the women enrolled in the trial. These exit interviews, which took place immediately after the first and second antepartum visits with their physicians and before the women in the intervention group received counseling, were conducted by a research assistant and took <2 minutes to complete. The interview included five questions about the content of the doctor's advice and two questions about what the woman agreed to do about her smoking behavior. The women's responses to the five questions about the content of the doctor's advice enabled us to see how closely the residents were adhering to the contents of the intervention and basic care protocols.

Third, the immediate effect of each resident's advice was also assessed from the exit interviews by comparing the responses of the women in the intervention and basic care groups to the two questions asking what they had agreed to do about changing their smoking behavior.

Long-term adherence to the intervention and basic care protocols was assessed by comparing the responses to the exit interviews conducted during the first year of the trial with those conducted in the second year.

Data analysis. The videotape scores and coded data from the exit interviews were entered into a personal computer. Paired Student t tests were used to compare the pretraining and posttraining videotape scores and times. χ^2 analyses of 2×2 contingency tables were used to examine the differences between the exit interview responses of the women in the intervention group compared with those in the basic care group, at the first or second antepartum visit.

This study was approved by the University of Vermont's Institutional Review Board.

Results

Comparisons of the pretraining and posttraining videotapes of the first 20 residents showed a significant effect for training on the content and style of the smoking cessation advice but not on the time taken to deliver the advice. The average score for the content and style of the advice increased from 2.15 (SD 0.88) before training to 6.05 (SD 0.69) after training, $t = -16.28$, $p = 0.0001$. The average time for delivery of the advice was 3.14 (SD 1.21) minutes before training and 3.09 (SD 0.82) minutes after training, a nonsignificant difference.

During the first 24 months of the trial, 250 women receiving their prenatal care through the Maternal-Infant Care clinic were enrolled into the trial, equally divided by random allocation between the intervention group and the basic care control group. Because this trial is still in progress and because some women have miscarried and others have transferred to private obstetric practices or have moved out of the area for their prenatal care, 103 women in the intervention group and 102 in the basic care group have been seen for their second antepartum visit. Of these, 18 women in the intervention group and three in the basic care group reported not smoking at this visit, leaving 85 smokers in the intervention group and 99 smokers in the basic care group for evaluation of advice to stop smoking or to cut down at the second antepartum visit.

The women's responses to the exit interviews after the first and second antepartum visits showed high

Table II. Exit interview responses about advice to stop smoking (number and percent of positive responses)

	First antepartum visit				*Second antepartum visit*			
	Intervention group (n = 125)		*Basic care group (n = 125)*		*Intervention group (n = 103)*		*Basic care group (n = 102)*	
Exit interview question	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
Did your doctor . . .								
. . . talk about smoking?	122	98	119	95	99	96	67	66*
. . . advise you to stop or stay quit?	120	96	115	92	94	91	53	52*

*$p = 0.0001$ for the 2 × 2 contingency tables comparing positive and negative responses of intervention and basic care groups for this question at this visit.

levels of adherence by the residents to both the intervention and basic care protocols. For the items, "Did your doctor talk about smoking?" and "Did your doctor advise you to stop smoking?", which address comments or advice common to both the intervention and basic care protocols, there were no significant differences at the first antepartum visit. However, at the second antepartum visit, when there were no prompts in the charts of women in the basic care group, there were substantial and highly significant differences, with fewer women in the basic care group reporting having their doctor talk about smoking or advise them to stop at this visit compared with the those in the intervention group (Table II).

For the items, "Did your doctor ask you to set a quit date?" and "Did you agree to stop?", there were highly significant differences between the two groups at both visits. Women in the intervention group were substantially more likely to report being asked to set a quit date (80%), a key part of the intervention protocol, and to agree to stop smoking (54%) at the first antepartum visit than were those in the basic care group (14% for each). Similar but less marked differences were seen at the second visit (Table III).

Responses to the three exit interview items concerning cutting down cigarette consumption also show significant differences between the two groups, with more women in the intervention group being asked to set cut down dates and agreeing to do so than those in the basic care group (Table III).

Levels of adherence to both the intervention and basic care protocols have remained remarkably steady during the course of the trial. The proportions of positive responses to the three exit interview items, "Did your doctor talk about smoking?", "Did your doctor advise you to stop smoking?", and "Did your doctor ask you to set a quit date?", did not differ significantly during the first and second years of the trial for the intervention group or for the basic care group (Table IV).

Comment

Comparison of the pretraining and posttraining videotapes clearly show that the residents changed the content and style of their advice after training but on average spent the same amount of time delivering this advice. After training the residents put substantially less emphasis on discussing the risks of smoking to the pregnancy, fetus, and mother and more emphasis on trying to gain a commitment to a smoking behavior change. Style also changed, with more of the residents asking open-ended questions after they were trained.

Adherence to the protocol by the residents has been good, as judged by the exit interviews of the women conducted immediately after their first two antepartum visits with their physicians. This level of adherence to both the intervention protocol and the basic care protocol, which has been maintained over the first 2 years of the trial, needs to be seen in the context of each obstetric resident doing one or two 2-month rotations on the prenatal service each year and each family practice resident doing a single 2-month rotation. Residents rarely see the same woman for more than two visits and may also be asked to fill in unexpectedly for their colleagues at short notice. The protocol prompt sheets that we devised for this study were modeled on those used by Solberg et al.[22] to prompt family physicians to provide smoking cessation advise[22] but also include the essential elements of the advice to be given. These prompt sheets play a key role in helping to maintain adherence to the protocol, because they show what progress the woman has made between visits and they also highlight the advice to be delivered at each visit. The prompt sheets are updated by a nurse interviewer and placed in the women's charts to cue the residents. Prompts have been used successfully by others to increase physicians' adherence to providing smoking cessation advice.[11, 13, 25, 28]

The third measure of effectiveness, gaining the woman's commitment or agreement to change her smoking behavior, also differed significantly between the inter-

Table III. Exit interview responses from current smokers about setting quit dates and cutting down (number and percent of positive responses)

	First antepartum visit				*Second antepartum visit*			
	Intervention group (n = 125)		*Basic care group (n = 125)*		*Intervention group (n = 85)*		*Basic care group (n = 99)*	
Exit interview question	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
Did your doctor...								
... ask you to set a quit date?	100	80	18	14*	63	74	7	7*
Did you agree to stop?	67	54	18	14*	32	38	6	6*
Did your doctor...								
... advise you to cut down?	50	40	73	58†	42	49	41	41
... ask you for a cut down date?	37	30	5	4*	37	44	7	7*
Did you agree to cut down?	35	28	8	6*	26	31	5	5*

*$p = 0.0001$ for the 2 × 2 contingency tables comparing positive and negative responses of intervention and basic care groups for this question at this visit.

†$p < 0.05$ for the 2 × 2 contigency tables comparing positive and negative responses of intervention and basic care groups for this question at this visit.

Table IV. Adherence to intervention and basic care protocols after 1 and 2 years of the trial (percent positive responses to each question)

	First antepartum visit							
	Intervention group				*Basic care group*			
	1 yr (n = 62)		*2 yr (n = 63)*		*1 yr (n = 64)*		*2 yr (n = 61)*	
Exit interview question	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
Did your doctor...								
... talk about smoking?	61	98	61	97	61	95	58	95
... advise you to stop?	59	95	61	97	57	89	58	95
... ask you to set a quit date?	49	79	51	81	9	14	9	15

There are no significant differences within groups.

vention and basic care groups. These differences, which were seen for agreeing to stop smoking and for agreeing to cut down cigarette consumption, indicate the importance of actively seeking the women's commitment to make a change in her smoking behavior before her next antepartum visit.

We recommend the inclusion of training in methods to deliver brief smoking cessation advice and, most importantly, information about office system components, such as prompt sheets, as part of every residency in obstetrics. Others have described the training of residents in family medicine and internal medicine[18, 23] and pediatrics.[23] The important elements of the brief advice to be given are well summarized in the National Cancer Institute's manual for physicians.[16] Whether physicians in established practice could be persuaded to follow a scripted protocol, such as we describe here, remains to be seen, but we think they could, because efforts by others to train family physicians and internists to follow specific elements of smoking cessation protocols have met with some success.[11-13]

The role of a nurse-counselor in the practice in supporting smoking behavior change should also be considered,[24] as there is evidence that the combination of advice from physicians and nonphysicians is more effective in promoting smoking cessation than advice from either alone.[19]

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Impact of histocompatibility antigens on pregnancy outcome

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OBJECTIVE: The role of histocompatibility antigens in pregnancy outcome is controversial. This controversy may be because the initial studies were of small numbers or the patient groups were not homogeneous. The purpose of this study is to clarify these discrepant results by carrying out histocompatibility antigen typing and mixed lymphocyte culture on couples with idiopathic recurrent spontaneous abortion and by comparing results with those of fertile couples.
STUDY DESIGN: Sixty couples with at least three spontaneous abortions and 60 normal couples with at least two successful pregnancies were included. Histocompatibility antigen typing and mixed lymphocyte culture were performed by using the standard techniques. The data were analyzed statistically with the Fisher exact test and χ^2.
RESULTS: Our results failed to show any difference between normal and aborting couples with regard to HLA-A, HLA-B, and HLA-DR distribution or sharing or to mixed lymphocyte culture responsiveness.
CONCLUSION: Our study, along with other studies, emphasizes that the histocompatibility antigen system does not have an impact on pregnancy outcome. (Am J Obstet Gynecol 1992;166:1364-9.)

Key words: Recurrent spontaneous abortion, human leukocyte antigen sharing, reproductive immunology

Successful reproduction in humans continues to be an immunologic enigma as the conceptus is a semiallogenic graft with partially foreign, paternal histocompatibility antigens. There are studies[1-4] in the literature suggesting that reproductive success is due to antigenic disparity between mating partners. This disparity is necessary so that the conceptus will be immunogenic and stimulate recognition to evoke a protective mechanism against expected immunologic destruction. The role of antigenic disparity, or incompatibility, has been examined by determining sharing of histocompatibility antigens (HLA) between wife and husband. The majority of these studies were done on couples with idiopathic recurrent spontaneous abortion. Several studies reported increased sharing of HLA-A and HLA-B antigens between the partners of these couples.[5-14] Furthermore, findings of hyporesponsiveness in maternal-paternal mixed lymphocyte culture implied that compatibility in the HLA-D locus may be the cause of recurrent spontaneous abortion.[15] These findings were supported by studies showing increased HLA-DR (serodefined antigens of the D locus) sharing in such couples.[11, 16, 17] Consequently, antigenic similarity (increased HLA sharing in any of the HLA loci) between husband and wife has also been proposed as a cause of recurrent spontaneous abortion. In recent literature, however, the number of studies refuting those findings has been increasing.[18-25] Therefore the role of the HLA system in the outcome of pregnancy is controversial. These inconsistencies may be explained by studies of patients with heterogeneous causes of recurrent spontaneous abortion or because of the limited size of some groups.

The current study was undertaken to carry out HLA-A, HLA-B, and HLA-DR typing and mixed lymphocyte culture on a large and homogeneous group with recurrent spontaneous abortion. These results were compared with those of normal couples with no history of spontaneous abortion. A compilation of studies from the literature is also presented.

Material and methods

Patients. Couples with at least three spontaneous abortions and no living children in both Colorado and Louisiana were investigated thoroughly to rule out anatomic abnormalities of the uterus and genetic, endocrinologic, infectious, and autoimmune diseases. Fifty-five white couples, four black couples, and one mixed (wife white, husband black) couple met the criteria and were included in the study.

Controls. The same number of couples were matched with the aborting couples for age and race. These couples had at least two successful pregnancies and no history of spontaneous abortion.

Histocompatibility testing. HLA-A, HLA-B, and HLA-DR typing was performed with the standard National Institutes of Health technique described by Terasaki et al.[26] with tissue typing trays obtained from One Lambda (Los Angeles). Gen Trak (Plymouth Meeting,

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Table I. HLA-A and HLA-B antigen frequencies (percent) in aborting and normal couples

HLA type in A and B loci	*Aborting (60 couples) (% frequency)*	*Normal (60 couples) (% frequency)*
HLA-A		
1	27.9	30.7
2	46.7	44.3
3	27.5	35.2
11	16.4	6.8
23	4.9	2.3
24	22.1	17.5
25	8.2	3.4
26	5.7	3.4
28	4.9	6.8
29	5.3	8.0
30	7.3	9.1
31	4.1	5.7
32	8.9	10.2
Blank	12.3	9.8
HLA-B		
7	22.1	20.5
8	22.1	20.5
13	5.7	5.7
14	7.4	6.8
18	8.2	11.4
27	8.2	9.1
35	22.1	23.9
37	2.5	2.3
38	4.1	2.3
39	5.7	4.5
41	0.0	2.3
44	22.1	26.1
49	3.3	1.1
50	2.5	2.3
51	4.9	5.7
52	0.8	2.3
53	3.3	4.5
55	2.5	2.3
57	4.1	6.8
60	6.6	9.1
61	4.6	4.5
62	16.4	13.6
Blank	9.0	7.5

Table II. HLA-DR antigen frequencies (percent) in aborting and normal couples

HLA type in DR locus	*Aborting (60 couples) (% frequency)*	*Normal (60 couples) (% frequency)*
HLA-DR		
1	22.1	18.2
2	26.2	23.9
3	25.4	21.6
4	38.6	39.8
5	14.8	18.2
6	11.5	12.5
7	18.0	25.0
8	7.4	6.5
9	4.9	0.4
10	2.5	1.1
11	11.4	11.4
13	2.8	3.4

Pa.), and Pel-Freeze (Brown Deer, Wis.). B Lymphocytes for DR typing were prepared with the nylon-wool method.[27]

Mixed lymphocyte culture. One-way mixed lymphocyte culture was performed with traditional methods. Two couples were tested at a time, one from the aborting group and one from the control group. A total of 15 different experiments were performed. Briefly, after purification of peripheral blood mononuclear cells, the samples were suspended at 1×10^6 in RPMI 1640 (GIBCO, Grand Island, N.Y.) containing 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mmol/L glutamine, and 10% pooled male serum. Stimulating cells were irradiated with a dose of 2000 rads to abolish their capacity to proliferate. Cultures were carried out in triplicate in round-bottomed microtiter trays (Linbro Chemical Company, New Haven, Conn.) with 100,000 responder and 100,000 irradiated stimulator cells in a volume of 200 μl. Media and autologous and pooled lymphocytes were used to be negative and positive controls. Cultures were incubated at 37° C with 100% humidity in a 5% carbon dioxide and air atmosphere for 5 days. Tritiated thymidine, specific activity 6.7 Ci/mmol (New England Nuclear, Boston) was then added, 1 μCi per well. After 12 hours of additional incubation, plates were harvested in a multiple harvester. The samples were then placed in vials containing scintillation fluid and the amount of radioactivity that was incorporated into the deoxyribonucleic acid of proliferating cells was determined by liquid scintillation counting.

The results of mixed lymphocyte culture were expressed as relative response and were calculated by

$$\text{Relative response} = \frac{\text{Test counts/min} - \text{Autologous counts/min}}{\text{Median response counts/min} - \text{Autologous counts/min}} \times 100$$

When the relative response ≥20, mixed lymphocyte culture was accepted to be positive in another way, stimulation occurred.

Statistical analysis. The data were analyzed with the Fisher exact test and χ^2 analysis.

Results

HLA class I and II antigens (HLA-A, HLA-B and HLA-DR). The distribution of HLA-A, HLA-B, and HLA-DR antigens was analyzed in both aborting and normal couples. The frequency of antigens is shown in Tables I and II. There was no significant difference between the two groups. Also, no association between recurrent spontaneous abortion and a particular HLA

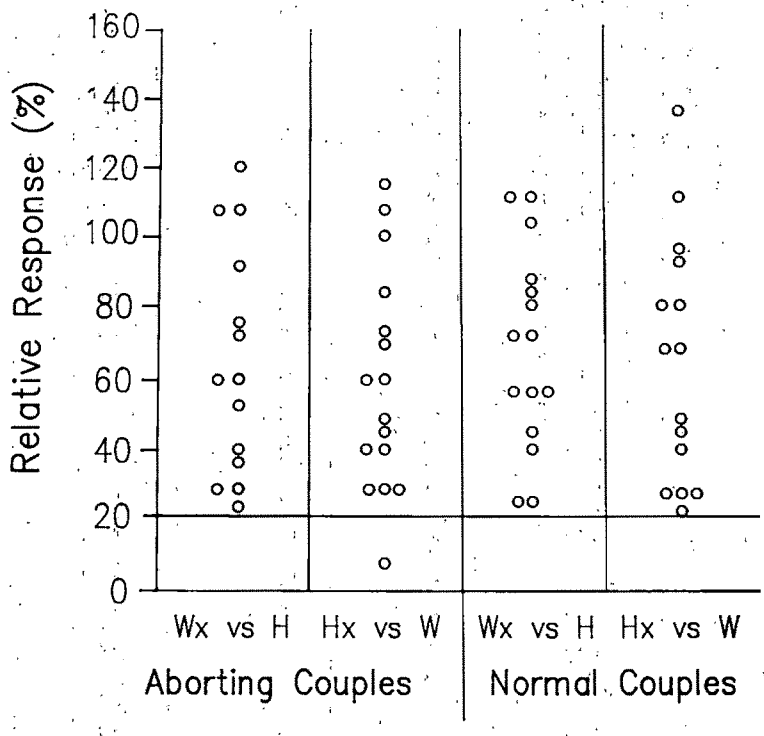


Fig. 1. Mixed lymphocyte culture in 15 aborting and 15 normal couples. Reactivity was expressed as relative response. Positive response was accepted when relative response ≥20. Wife *(Wx)* versus husband *(H);* wife's irradiated cells as stimulator, husband's cells as responder; husband *(Hx)* versus wife *(W);* husband's irradiated cells as stimulator, wife's cells as responder.

Table III. Number of HLA-A, HLA-B, and HLA-Dr antigens shared by 60 normal and 60 aborting couples

No. of antigens shared	*Aborting couples*		*Normal couples*		*p**
	No. and % sharing	*No. and % not sharing*	*No. and % sharing*	*No. and % not sharing*	
A,B loci					
0	24 (40.0%)	36 (60.0%)	22 (36.7%)	38 (63.3%)	0.851
1	25 (41.7%)	35 (58.3%)	34 (56.7%)	26 (43.3%)	0.144
2	10 (16.7%)	50 (83.3%)	4 (6.7%)	56 (93.3%)	0.153
3	1 (1.7%)	59 (98.3%)	0 (0.0%)	60 (100.0%)	1.000
4	0 (0.0%)	60 (100.0%)	0 (0.0%)	60 (100.0%)	1.000
DR locus					
0	45 (75.0%)	15 (25.0%)	36 (60.0%)	24 (40.0%)	0.118
1	15 (25.0%)	45 (75.0%)	23 (38.3%)	37 (61.7%)	0.169
2	0 (0.0%)	60 (100.0%)	1 (1.7%)	59 (98.3%)	1.000
A,B, and DR loci					
0	18 (30.0%)	42 (70.0%)	12 (20.0%)	48 (80.0%)	0.292
1	24 (40.0%)	36 (60.0%)	33 (55.0%)	27 (45.0%)	0.143
2	15 (25.0%)	45 (75.0%)	12 (20.0%)	48 (80.0%)	0.663
3	3 (5.0%)	57 (95.0%)	2 (3.3%)	58 (96.7%)	0.493
4	0 (0.0%)	60 (100.0%)	0 (0.0%)	60 (100.0%)	
5	0 (0.0%)	60 (100.0%)	0 (0.0%)	60 (100.0%)	
6	0 (0.0%)	60 (100.0%)	0 (0.0%)	10 (16.0%)	

*Fisher's exact test.

antigen was found. Furthermore, no excess of maternal or paternal homozygosity was observed.

The number of shared HLA antigens in both groups is shown in Table III. To accentuate the distinction between aborting and normal couples, the data were analyzed separately for each locus and for all the loci together. As illustrated, the results for each locus were not different from those found with normal couples. In a combined analysis of HLA-A, HLA-B and HLA-DR, 30% (18/60) aborting couples did not share any antigen, 40% (24/60) shared one antigen, 25% (15/60) two antigens, and only 5% (3/60) had three antigens

Table IV. Prevalence of shared HLA antigens at the A, B, and DR loci in aborting and normal couples

	Aborting couples			*Normal couples*		
Investigation	*HLA-A*	*HLA-B*	*HLA-DR*	*HLA-A*	*HLA-B*	*HLA-DR*
Group A						
Komlos et al.[5] (1977)	11/22 (50.0%)	8/22 (36.4%)	ND	3/7 (42.9%)	1/7 (14.3%)	ND
Gerencer[6] (1979)	20/45 (44.4%)	17/45 (37.8%)	ND	20/79 (25.3%)	20/79 (25.3%)	ND
Schacter[8] (1979)	12/39 (30.8%)	9/39 (23.1%)	ND	4/17 (23.5%)	2/17 (11.8%)	ND
Taylor and Faulk[9] (1981)	4/4 (100.0%)	3/4 (75.0%)	3/4 (75.0%)	ND	ND	ND
Aoki (1982)	20/26 (76.9%)	9/26 (34.6%)	22/26 (84.6%)	26/45 (57.8%)	10/45 (22.2%)	11/45 (24.4%)
Beer et al.[11] (1983)	9/10 (90.0%)	3/10 (30.0%)	4/10 (40.0%)	2/16 (12.5%)	0/16 (0.0%)	4/16 (25.0%)
Unander and Olding[7] (1983)	7/8 (87.5%)	1/8 (12.5%)	7/8 (87.5%)	ND	ND	ND
McIntyre and Faulk[10] (1983)	8/20 (40.0%)	3/20 (40.0%)	7/20 (35.0%)	5/12 (41.7%)	5/12 (41.7%)	1/12 (8.3%)
Johnson et al.[12] (1984)	17/28 (60.7%)	11/28 (39.3%)	7/14 (50.0%)	21/41 (51.2%)	10/41 (24.4%)	6/20 (30.0%)
Thomas et al.[13] (1985)	9/21 (42.9%)	8/21 (38.1%)	6/21 (28.6%)	9/21 (42.9%)	5/21 (23.8%)	3/21 (14.3%)
Coulam et al.[14] (1987)*						
Group B						
Lauritsen et al.[18] (1976)	12/29 (41.4%)	6/29 (20.7%)	ND	23/35 (65.7%)	11/35 (31.4%)	ND
Caudle et al.[19] (1983)	3/12 (25.0%)	3/12 (25.0%)	2/12 (16.7%)	44/77 (57.1%)	21/77 (27.3%)	ND
Oskenberg et al.[20] (1983)	11/33 (33.3%)	7/33 (21.2%)	ND	13/30 (43.3%)	9/30 (30.0%)	ND
Nordlander et al.[24] (1983)	6/14 (42.9%)	6/14 (42.9%)	5/14 (35.7%)	22/40 (55.0%)	15/40 (37.5%)	20/40 (50.0%)
Cauchi et al.[21] (1988)	32/83 (38.6%)	21/83 (25.3%)	36/83 (43.4%)	52/100 (52.0%)	41/100 (41.0%)	38/100 (38.0%)
Sargent et al.[22] (1988)	10/18 (55.6%)	7/18 (38.9%)	8/18 (44.4%)	7/10 (70.0%)	4/10 (40.0%)	4/10 (40.0%)
Christiansen et al.[25] (1989)	30/56 (53.6%)	18/56 (32.1%)	25/56 (44.6%)	20/33 (60.6%)	11/33 (33.3%)	13/33 (39.4%)
Smith et al.[23] (1988)†	115 Patients were studied					
Eroglu et al. (1990)	24/60 (40.0%)	19/60 (31.7%)	15/60 (25.0%)	26.60 (43.3%)	16/60 (26.7%)	24/60 (40.0%)

ND, Not done.

*Found an association between DQ locus and primary recurrent spontaneous abortion.

†Analysis of results was done differently in this study.

in common. Those percentages in normal couples were, respectively, 20%, 55%, 20%, and 3.33%.

The HLA sharing data from this study and from other published data are presented in Table IV. For the purposes of classification, the results are divided into group A, those studies that reported increased HLA sharing in aborting couples, and group B, those that did not. As can be seen, the number of studies reporting increased HLA sharing (group A) is slightly more than those who did not, but the number of cases in group B is more than those studies of group A.

Mixed lymphocyte culture—stimulation indices. The results of one-way mixed lymphocyte culture are shown in Fig. 1. All cultures showed normal stimulation except for that of couple 12, which showed a low level of stimulation when the wife's cell was responder to the husband's cells and to third party cells. Female lymphocytes from couples with recurrent spontaneous abortion stimulated and responded equally to cells both from their husbands' cells and third parties' cells (husbands of control groups). Similarly, cells from husbands of couples with recurrent spontaneous abortion were able to stimulate and respond to cells from their wives and the control group. When the relative response of couples with recurrent spontaneous abortion was compared with normal couples, there was no difference between the two groups.

Comment

The data presented here failed to show an increased incidence of antigen sharing between the partners of couples with recurrent spontaneous abortion when compared with controls. Furthermore, the distribution of HLA, HLA-A, HLA-B, or HLA-DR antigens in couples with recurrent spontaneous abortion was very similar to that of control group.

Increased sharing of HLA-A and HLA-B antigens in couples with recurrent spontaneous abortion was first reported by Komlos et al.[5] in 1977 (Table IV); similar findings were reported by other groups.[8-14] Other reports suggested that sharing of class II antigens (HLA-D) was even more significant than sharing in class I (HLA-A, HLA-B, HLA-C) antigens.[11, 16, 17] These findings of increased HLA sharing have been interpreted both genetically and immunologically to explain the etiology of idiopathic recurrent spontaneous abortion. The immunologic interpretation suggests that an immune response is necessary for proper im-

plantation and maintenance of pregnancy. The genetic interpretation postulates that the gene associated with fetal wastage is a recessive lethal gene linked to an HLA locus and that linkage disequilibrium exists between these loci.[13] It appears, however, from our results that increased HLA sharing has no relation to recurrent spontaneous abortion. Twenty-three percent of our normal couples shared two to three antigens without any history of spontaneous abortion. Obert et al.[28] reported an association between increased HLA sharing and idiopathic recurrent spontaneous abortion in Hutterites,* who have increased inbreeding. The authors also, however, reported that sharing HLA antigens does not preclude normal pregnancy. In agreement with our findings, the association between recurrent spontaneous abortion and increased HLA sharing was not found by other investigators.[18-25] This contradiction may involve a number of variables, including the increased number of split HLA antigens (subgroups of core antigens, i.e., HLA-A10 was split to be HLA-A25 and A26, etc.), sample size, and lack of homogeneity of the population studied. We hoped to eliminate these variables in this report by using a relatively large homogeneous group. Furthermore, we used split antigens, not core antigens.

Mixed lymphocyte culture is one of the methods to investigate allograft reactions in vitro and has been used by some investigators in an attempt to detect feto-maternal immunologic interactions. In contrast to previous reports of depressed mixed lymphocyte culture responses[8-11, 15, 20] between wives and husbands of aborting couples, our studies failed to demonstrate a depression of mixed lymphocyte responsiveness in such couples. When results were compared with normal couples, the relative response did not deviate from that of the control group. Thus our findings are in agreement with Thomas et al.[15]

Although our study has resulted in negative findings, we are in agreement with recent literature emphasizing that the status of the HLA system does not have a major impact on pregnancy outcome or that traditional typing and mixed lymphocyte culture are not the right criteria to sufficiently define the role of incompatibility in human reproductive performance.

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*The Hutterites, an Anabaptist religious isolate, live in colonies (communal farms) composed of 100 to 150 people. Marriages are leut-endogomous.

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Changes in human fetal cerebral hemoglobin concentration and oxygenation during labor measured by near-infrared spectroscopy

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OBJECTIVE: The purpose of this study was to measure by near-infrared spectroscopy changes in human fetal cerebral oxyhemoglobin, deoxyhemoglobin, and cerebral blood volume during labor and to calculate mean cerebral hemoglobin oxygen saturation.
STUDY DESIGN: The effects of uterine contractions with and without fetal heart rate decelerations were compared in eight singleton term fetuses. Results were analyzed by analysis of variance.
RESULTS: In six of eight fetuses normal uterine contractions were associated with proportional decreases in both oxyhemoglobin and deoxyhemoglobin and a fall in cerebral blood volume without desaturation of cerebral hemoglobin. Contractions with fetal heart rate decelerations produced different results in that oxyhemoglobin fell but deoxyhemoglobin rose, indicating cerebral desaturation. In two of the eight fetuses normal contractions were associated with increases in oxyhemoglobin, deoxyhemoglobin, and cerebral blood volume; no decelerations were seen in either fetus. Mean cerebral hemoglobin oxygen saturation calculated during normal contractions was 43% ± 10% (SD).
CONCLUSION: Uterine contractions were associated with detectable changes from baseline in cerebral oxyhemoglobin, deoxyhemoglobin, and cerebral blood volume. (Am J Obstet Gynecol 1992;166:1369-73.)

Key words: Near-infrared spectroscopy, fetal brain oxygenation, labor, uterine contraction, fetal heart rate deceleration

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No current techniques allow the direct assessment of fetal cerebral oxygenation during labor, although information can be obtained about the oxygenation and acid-base status of the fetal scalp.[1-4] Because of the relative transparency of biologic tissue to light in the near-infrared region of the spectrum (700 to 1000 nm), it is possible to transmit near-infrared light through ≤8 cm of tissue to obtain spectroscopic information noninvasively from the brain. The technique of near-infrared spectroscopy has been used to provide quanti-

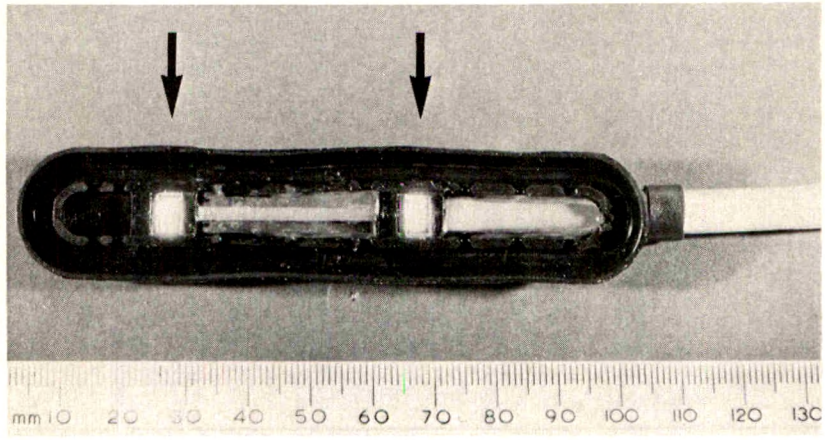


Fig. 1. Two glass prisms *(arrows)* held at a constant separation from each other by the black silicone rubber molding.

Table I. Details of labor and delivery in eight fetuses

Maternal age (yr)	28 (22-35)*
Parity	
Primiparous	8
Multiparous	0
Onset of labor	
Spontaneous	5
Induced	2
Stimulated	1
Duration of labor (hr)	8.75 (6.75-12.5)*
Epidural	7
Augmented with oxytocin	8
Mode of delivery	
Normal vaginal	3
Forceps	3
Cesarean	2
Cord blood pH <7.2	5

*Median and range. All other values given are number of fetuses.

tative data on cerebral oxygenation and hemodynamics in the newborn infant.[5-7] We adapted this technique to measure changes in fetal cerebral hemoglobin concentration, oxygenation, and blood volume during labor. The purpose of our study was to examine the effects of uterine contractions with and without fetal heart rate decelerations on these variables and to measure fetal mean cerebral hemoglobin oxygen saturation.

Material and methods

We studied eight singleton term fetuses of gestational age 39 to 42 (median 40) weeks and birth weight of 2.6 to 4.2 (median 3.6) kg during labor, the details of which are given in Table I. Observations were recorded continuously for a period of 169 to 343 (median 208) minutes until 360 to 0 (median 140) minutes before delivery occurred. Near-infrared spectroscopy was continued in two fetuses throughout the second stage until delivery. In seven fetuses the postnatal course was uncomplicated, but one infant required a short admission to the neonatal unit for observation because of acidemia at birth. The project was approved by the University College London Faculty of Clinical Sciences Committee on the Ethics of Clinical Investigation, and written maternal consent was obtained.

The technique of near-infrared spectroscopy depends on absorption by the chromophores oxyhemoglobin and deoxyhemoglobin of near-infrared light transmitted through the fetal head. The modified Beer-Lambert law describing optic absorption in a highly scattering medium may be expressed as OD = acLB + G, where OD is the tissue attenuation (optical densities), a is the absorption coefficient of the chromophore (millimoles per liter per centimeter), c the concentration of chromophore (millimoles per liter), L the distance between the points where light enters and leaves the tissue (centimeters), B a path-length factor that takes account of the scattering of light in the tissue (which causes the optic path-length to be greater than L), and G a factor related to the geometry of the tissue. If during a study L, B, and G remain constant, changes in chromophore concentration can be calculated from the change in absorption divided by the product of aLB.[8] The absorption coefficients of oxyhemoglobin and deoxyhemoglobin are known,[9] and a mean value for B of 4.39 (SD 0.28) has been obtained.[10]

Fiberoptic bundles that conveyed light to and from the fetal head were introduced through the cervix after it had dilated to 3 to 6 (median 4) cm after rupture of the amniotic membranes. The ends of the bundles were held 3 cm apart in a specially designed silicone rubber molding (Fig. 1) placed against the side of the fetal head during routine vaginal examination. Coupling between the fiber ends and the fetal scalp was maintained

by maternal tissue pressure. No significant difficulty in placement was encountered, and no maternal discomfort was apparent. All the babies were examined after delivery, and no skin marking caused by the molding was found. The fiber bundles conveyed near-infrared light to a spectrophotometer (NIR 500 or 1000, Hamamatsu Photonics KK, Hamamatsu City, Japan) that detected changes in attenuation at three or six different wavelengths (NIR 500 or 1000, respectively) between 775 and 908 nm. These were converted into concentration changes of oxyhemoglobin and deoxyhemoglobin with a previously established algorithm.[9,10] Changes in total cerebral hemoglobin concentration were obtained as the sum of changes in oxyhemoglobin and deoxyhemoglobin, and changes in cerebral blood volume were calculated from cord hemoglobin concentration and changes in total cerebral hemoglobin concentration, assuming a cerebral/large-vessel hematocrit ratio of 0.69.[7] Fetal heart rate (FHR) and uterine activity were monitored continuously with a cardiotocograph (Hewlett Packard 80300A, Hewlett Packard, Andover, Mass.). All data were recorded simultaneously onto magnetic disk for subsequent analysis.

Results

Data were obtained without difficulty from all eight fetuses. Observations were available during 82 contractions, of which 20 were associated with FHR decelerations (a fall in FHR of ≥15/min for ≥15 seconds).

During normal contractions (n = 44) in six of the eight fetuses, oxyhemoglobin and deoxyhemoglobin fell from the baseline, the mean fall being 0.50 ± SD 0.27 μmol/100 gm brain tissue and 0.78 ± 0.60 μmol/100 gm, respectively (Figs. 2, *a*, and 3). Total hemoglobin concentration fell by 1.15 ± 0.60 μmol/100 gm and cerebral blood volume by 0.70 ± 0.39 ml/100 gm. The changes in cerebral hemoglobin concentrations occurred simultaneously with contractions. Twenty early FHR decelerations were observed in five of these six fetuses. As with normal contractions, the FHR decelerations were associated with a fall in oxyhemoglobin of 0.87 ± 0.54 μmol/100 gm; in contrast, deoxyhemoglobin always rose (Figs. 2, *b*, and 3) by 1.1 ± 0.60 μmol/100 gm ($p < 0.01$ vs change in deoxyhemoglobin in normal contractions, analysis of variance), indicating transient cerebral desaturation.

In the remaining two fetuses a different pattern was observed during normal contractions (n = 18), with a consistent rise in both oxyhemoglobin and deoxyhemoglobin of 0.71 ± 0.43 μmol/100 gm and 1.22 ± 0.55 μmol/100 gm, respectively. Total hemoglobin concentration rose by 1.86 ± 0.95 μmol/100 gm and cerebral blood volume by 0.97 ± 0.52 ml/100 gm. No decelerations were observed in these two fetuses.

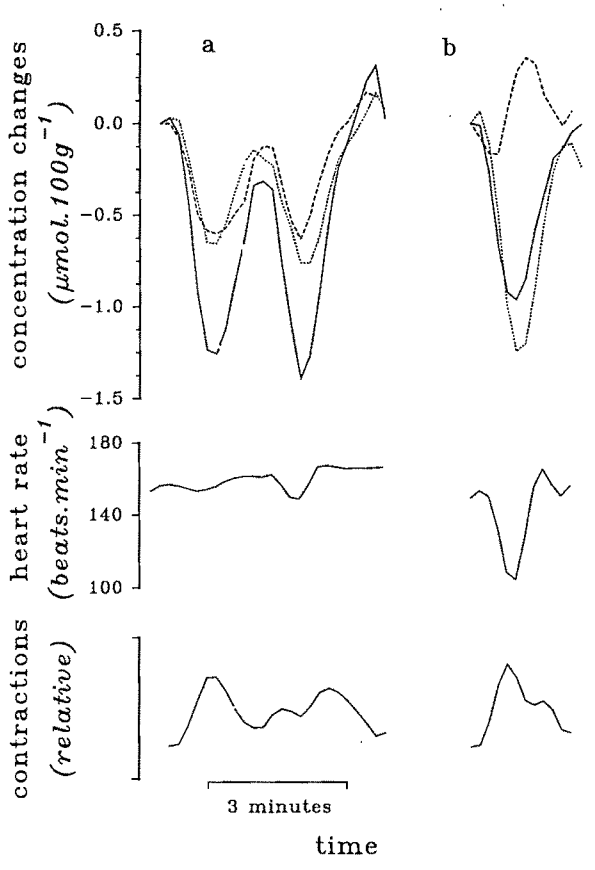


Fig. 2. Changes from an arbitrary baseline in the cerebral concentrations of oxyhemoglobin *(dotted line)*, deoxyhemoglobin *(dashed line)*, and total hemoglobin *(solid line)* in the same fetus during two normal contractions **(a)** and a contraction with an early FHR deceleration **(b)**.

In seven fetuses mean cerebral hemoglobin oxygen saturation was calculated during 14 normal contractions (range 1 to 3 per fetus, median 2) where oxyhemoglobin and deoxyhemoglobin changed in parallel. With the formula Mean cerebral hemoglobin oxygen saturation percent = 100 × Change in concentration of oxyhemoglobin/Change in concentration of oxyhemoglobin + deoxyhemoglobin, an estimate for mean cerebral hemoglobin oxygen saturation of 43% ± 10% (SD) was obtained. In one of the two fetuses in whom observations were continued until delivery, mean cerebral hemoglobin oxygen saturation remained in the range of 50% to 64%, the cord arterial oxygen tension was 2.0 kPa, and the pH_a 7.31. In the other, however, mean cerebral hemoglobin oxygen saturation fell from 35% ± 2% to 1% ± 3% (Fig. 4); in this infant the cord arterial oxygen tension was 1.2 kPa and pH_a 7.17.

Comment

These data describe the effects of uterine contractions with and without fetal heart rate decelerations on

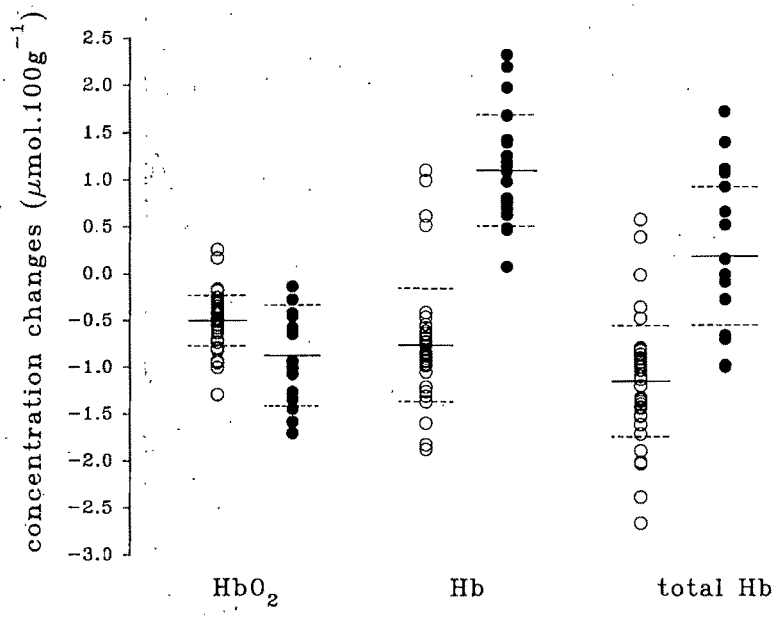


Fig. 3. Data from six fetuses showing maximum changes in cerebral oxyhemoglobin, deoxyhemoglobin, and total cerebral hemoglobin concentration during normal uterine contractions *(open circle)*, and those with FHR decelerations *(solid circle)*. Mean values ± SD are indicated.

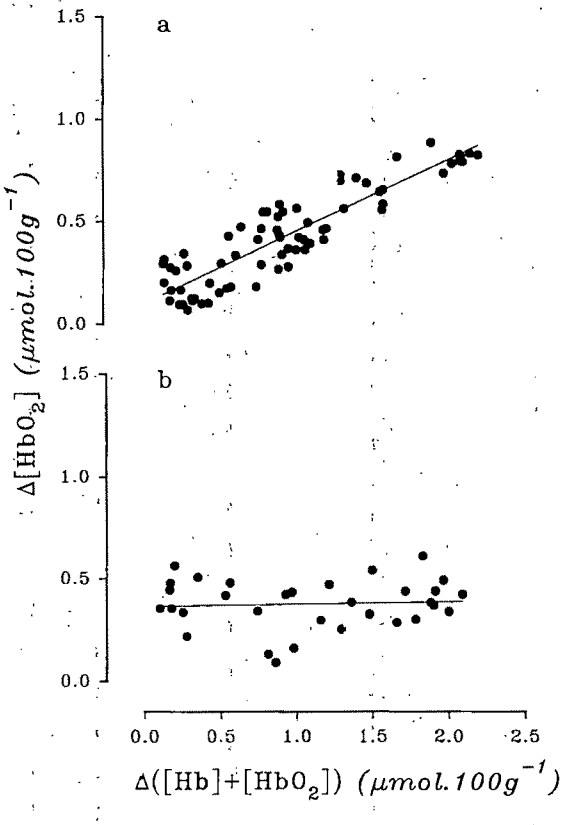


Fig. 4. Change in oxyhemoglobin and change in oxyhemoglobin plus deoxyhemoglobin during the first and second stages of labor in one fetus. Each point represents a 10-second collection of data during three consecutive contractions 165 to 141 minutes **(a)** and 23 to 18 minutes **(b)** before delivery. Mean cerebral hemoglobin oxygen saturation calculated from the least-mean-squares regression was 35% ± 2% **(a)** and 1% ± 3% **(b)**.

human fetal cerebral hemoglobin concentration, blood volume, and oxygenation. Comparison with other reports is difficult because there are no previous data from human studies and no animal experiment has measured similar hemodynamic variables. However, the fall in cerebral blood flow[11] and cerebral oxygen consumption[12] associated with head compression in fetal sheep suggests that the change in total hemoglobin concentration observed during normal uterine contractions probably reflected a balance between direct pressure effects, reducing cerebral blood flow and obstruction of venous drainage. The usual change in cerebral blood volume observed with normal contractions implied a fall in total cerebral blood volume of 30%, assuming neonatal values for cerebral blood volume.[7] Fetal heart rate decelerations were uniformly associated with transient cerebral desaturation, which was presumably caused by a reduction in cerebral oxygen delivery. Although we cannot rule out an effect on our results of a small change in the optical path-length during contractions, any such change could not have caused the large alterations in variables reported here. In the future continuous measurement of path-length will be possible.[13]

Our study reports the first measurements of fetal mean cerebral hemoglobin oxygen saturation. The measurements were made during contraction-related changes in total cerebral hemoglobin concentration when oxyhemoglobin and deoxyhemoglobin rose or fell in constant proportion, indicating that global saturation remained unchanged during the contraction. As such the measurement represented an average of

oxygen saturation throughout the brain. The relation between mean cerebral hemoglobin oxygen saturation and arterial or venous oxygen saturation is thus not necessarily linear; changes in the volume of a vascular compartment will affect the average saturation. However, changes in compartmentalization of blood during a measurement would cause the relation between oxyhemoglobin and deoxyhemoglobin to be nonlinear and this can be detected by near-infrared spectroscopy. Also, significant falls in cerebral oxygen delivery will be observed (as in our study) because the fall in global saturation is bigger than any possible effect of a change in compartment size.

The need for a method to assess fetal brain oxygenation has been previously emphasized.[14] At present the degree of hypoxemia to which a fetus is exposed during labor can be assessed by measuring acid-base status from intermittent scalp blood samples[2] or retrospectively from the umbilical cord blood, or by attempting to measure the pH,[3] oxygen tension,[4] or saturation[1] of the fetal scalp blood. The technique of near-infrared spectroscopy has several features that recommend it for intrapartum monitoring of the fetus. It is the first technique to allow continuous bedside monitoring of changes in human fetal cerebral hemoglobin concentration and oxygenation during labor. As an optic method it does not require electric coupling with the skin. Unlike scalp pulse oximetry, near-infrared spectroscopy directly interrogates the brain and is not dependent on the presence of an arterial pulsation. Near-infrared spectroscopy should supplement the present methods available for diagnosing fetal compromise in labor, such as FHR monitoring and scalp blood pH studies, and finally lead to a better understanding of the pathophysiologic characteristics of intrapartum cerebral hypoxemia.

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Risk factors for small-for-gestational-age birth in a preterm population

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OBJECTIVE: The objective of this study was to discover any distinct risk factors for small-for-gestational-age birth among premature infants.
STUDY DESIGN: Demographic and obstetric risk factors were compared for 136 small-for-gestational-age prematures and 636 appropriate-for-gestational-age premature infants.
RESULTS: Three significant risk factors for growth retardation among premature infants were found: black maternal race (odds ratio 2.2; 95% confidence interval (1.4 to 3.5); maternal toxemia (odds ratio 3.2; 95% confidence interval 1.7 to 6.1); and either low maternal weight gain (odds ratio 4.0; 95% confidence interval 1.8 to 8.8) or missing information on maternal weight gain, which could be a marker for late or no prenatal care (odds ratio 4.9; 95% confidence interval 1.9 to 12.6). Maternal smoking rates were similar in the small- and appropriate-for-gestational-age groups (42% and 43%, respectively).
CONCLUSIONS: Toxemia, weight gain, and race are likely risk factors for small-for-gestational-age birth in both preterm and term populations; within the already high-risk domain of prematurity, maternal smoking did not appear to confer added risk for small-for-gestational-age birth. (Am J Obstet Gynecol 1992;166:1374-8.)

Key words: Infant, small-for-gestational-age; infant, premature; infant, low-birth-weight

The incidence of intrauterine growth retardation has been estimated at 20% to 41% among preterm infants (infants born before 37 weeks' gestation).[1-3] Premature small-for-gestational-age (SGA) infants, when compared with appropriate-for-gestational-age (AGA) infants of similar gestational age, have been found to more frequently have low Apgar scores and to be more likely to require resuscitation at birth.[3] Additionally, such SGA preterm infants have been noted to be at increased risk for apnea, seizures, and neonatal death.[3] Heinonen et al.[3] have noted that the neonatal course of such infants is more likely to be complicated. In a population-based study in Finland they found that among preterm SGA infants 98% (48 of 49) had complications of some type during the neonatal period as compared with 78% (53 of 68) AGA premature infants.

Although risk factors for the occurrence of term SGA have been examined in the literature,[4, 5] risks for preterm SGA birth have been less frequently investigated. Hack and Fanaroff,[1] in a study of 182 very-low-birth-weight infants admitted to a neonatal intensive care unit (35 SGA and 147 AGA), noted that pregnancy-related hypertension was positively associated with SGA birth. Heinonen et al.[3] in a study of 117 preterm infants (49 SGA, 68 AGA) noted that the occurrence of preterm SGA was associated with maternal essential hypertension and toxemia.

In this paper we evaluate potential risk factors associated with the occurrence of preterm SGA birth among very-low-birth-weight infants and note how these risk factors compare with established risks for term SGA birth.

Methods

To recruit subjects for the National Collaborative Study of Patent Ductus Arteriosus, 3490 very-low-birth-weight babies were monitored at 13 clinical centers. All monitored babies weighed between 500 and 1750 gm at birth and had an expected viability of at least a few hours. Demographic information and details regarding the course of pregnancy and delivery were obtained from medical records and maternal postpartum interviews. Further details regarding data collection for this collaborative study have been reported elsewhere.[6]

Gestational age was determined by physical examination of the infant, according to the method of Dubowitz et al.[7] The gestational age for this study population was restricted to 30 and 31 weeks as a consequence of the weight criteria for inclusion in the collaborative study (500 to 1750 gm). For some gesta-

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tional ages, excluded babies <500 gm would more likely be SGA than AGA; for other gestational ages, excluded babies >1750 gm would more likely be AGA than SGA. To minimize this differential exclusion of SGA or AGA babies, we restricted the study to those gestational ages where few babies were excluded from the study base either because of low (<500 gm) or higher (>1750 gm) birth weights. We defined those gestational ages as ones where birth weight distributions became near normal and assessed this criterion by the comparability of the median and mean birth weights. For the gestational ages of 30 and 31 weeks, the median sex-specific birth weight exceeded the mean sex-specific birth weight by only 9 to 34 gm.

Babies were categorized as SGA if their birth weight was <10th percentile of gender-specific weights given by Williams et al.[8] All other babies were categorized as AGA. For 30 weeks' gestation the 10th percentile values for males and females were 1007 and 902 gm, respectively; for 31 weeks' gestation the values were 1159 and 1041 gm.

Rates of SGA were calculated for each gestational age group. Crude comparisons between the SGA and AGA groups were then made along a number of maternal demographic and medical variables.

Adjusting only for infant gender and gestational age, the effect of race was evaluated by means of a logistic model. Effects of other maternal demographic characteristics and variables describing the pregnancy and delivery were then examined by adding each variable in turn to the logistic model that already included variables for gestational age, gender, and race. Finally, a more fully adjusted model was constructed in which a number of potential risk factors were simultaneously included in the model. For all the logistic regressions, the effects are summarized by odds ratios and 95% confidence intervals.

Results

Data were evaluated from 772 singleton, premature, very-low-birth-weight babies, 136 SGA babies, and 636 AGA babies. The overall rate of SGA in this premature population was 17.6%. This rate was similar across the two gestational age categories (17.1% at 30 weeks' gestation and 18.2% at 31 weeks, Table I). Although the mean birth weight varied by gestational age, the mean difference between SGA and AGA birth weights was relatively uniform across the two gestational age categories, with SGA infants weighing about 400 gm less than AGA infants.

Other characteristics of the SGA and AGA groups are given in Table II. SGA and AGA babies differed in race and a number of other maternal characteristics, with the largest crude differences being in education, prepregnancy weight, weight gain, multivitamin and iron supplementation use, toxemia or other hypertension, and type of labor (spontaneous or not). There were only small differences between the SGA and AGA babies in maternal age and maternal smoking during this pregnancy. The individual and combined effects of these factors were evaluated with a series of logistic regressions. Seven factors were significantly related to the risk of preterm SGA birth in simple models that adjusted for only gestational age, gender and race: toxemia, other hypertension, maternal weight gain, prepregnancy weight, multivitamin and iron supplementation use, maternal employment, and type of labor. However, only race, toxemia, and gaining ≤5 pounds during the pregnancy or having no information about weight gain available remained significantly associated with preterm SGA in the more fully adjusted model (Table III).

Table I. SGA status by gestational age

	Gestational age		
	30 wk (n = 414)	*31 wk (n = 358)*	*Overall (n = 772)*
SGA			
No.	71	65	136
Birth weight (gm)			
Mean	858	990	921
Minimum	510	600	510
Maximum	1006	1150	1150
AGA			
No.	343	293	636
Birth weight (gm)			
Mean	1267	1392	1324
Minimum	907	1050	907
Maximum	1740	1750	1750
Rate of SGA	17.1%	18.2%	17.6%
Mean SGA − mean AGA	−409	−402	−403

Comment

Low birth weight can result from either a short gestation (preterm birth), growth retardation (SGA birth), or both. We have previously argued that these separate routes to low birth weight must be studied separately to discover any distinct risk factors.[5, 9]

In this study we have focused on SGA birth among premature infants, comparing the prenatal experiences of preterm SGA babies with those of preterm AGA babies. We compared the two groups in the largest study to date along 23 demographic, medical, and obstetric factors (Table II). Although there were a number of crude differences between the groups, after adjusting for the joint effects of all of these factors, the risk of SGA among preterm births increased independently with only three factors: black maternal

Table II. Maternal characteristics of SGA and AGA groups

	SGA (n = 136)	*AGA (n = 636)*
Demographic factors		
Race (% black)	57	35
Maternal age		
Mean (yr)	24	24
<20 (%)	31	26
Maternal education <12 yr (%)	43	33
Maternal unemployment (%)	12	9
Cigarette smoking during pregnancy (%)	42	43
Medications during pregnancy (%)		
Aspirin	10	7
Acetaminophen	33	35
Antihistamines	6	7
Antibiotics	10	14
Obstetric factors		
Maternal prepregnant weight		
Mean (lb)	134	127
≤119 lb (%)	28	37
120-159 lb (%)	37	45
≥160 lb (%)	15	9
Missing (%)	20	10
Parity (≥4)	28	22
Prior abortions (%)	44	38
Weight gain		
Mean (lb)	17	19
≤5 lb (%)	11	4
>5, ≤10 lb (%)	12	11
>10, ≤15 lb (%)	16	20
>15 lb (%)	34	53
Missing (%)	27	11
Multivitamin use (%)	71	84
Iron supplements used (%)	57	68
Toxemia (%)	22	8
Other hypertension (%)	7	3
Anemia (%)	8	10
Eclampsia (%)	1	1
Diabetes (%)	1	2
Venereal disease (%)	3	1
Urinary tract infection (%)	7	12
No spontaneous onset of labor (%)	29	19

race (odds ratio 2.2, 95% confidence interval 1.4 to 3.5), the incidence of maternal toxemia during the pregnancy (3.2, 1.7 to 6.12), and maternal weight gain, specifically the categories of ≤5 pounds during the pregnancy (4.0, 1.8 to 8.8) and no available information about maternal weight gain (4.9, 1.9 to 12.6). Previous studies have reported similar crude associations in preterm infants.[1, 3] For example, Hack and Fanaroff[1] reported rates of pregnancy-related hypertension of 26% for SGA mothers and 8% for AGA mothers; Heinonen et al.[3] reported rates of toxemia and hypertension of 41% for SGA mothers and 7% for AGA mothers. These numbers are similar to the rates found in this study, 26% toxemia or hypertension for SGA mothers versus 11% for AGA mothers. However, neither previous study reported results from multivariate models adjusting for a range of potential confounding factors.

The risk factors we found for SGA birth among prematures (toxemia, maternal weight gain, and race) are also risk factors for SGA birth among term babies.[4, 10] Growth retardation as a consequence of toxemia has been frequently reported.[4, 10] The role of low maternal weight gain is more problematic; low maternal weight gain may be a cause of growth retardation or another factor may influence both growth retardation along with factors related to weight gain. We interpret the high risk of SGA associated with missing information about weight gain to suggest that this unavailability is a likely marker for poor or late prenatal care, situations where weight gain information would not be routinely available. As for the role of race, although it consistently predicts adverse birth outcomes,[4, 11, 12] it may not do so in a strictly causal fashion. Black race may be a marker for a residual cluster of "bad experiences," social, economic, behavioral, and medical, that cause SGA.

Cigarette smoking during the pregnancy has been found to be a major risk factor for term SGA birth.[4, 12, 13] We did not find it to be a risk factor for SGA birth among premature infants. Other studies of SGA among preterm infants either reported no information about smoking[1] or reported only that their numbers were too small to evaluate its effect.[3] In our study 328 women reported smoking during pregnancy. However,

Table III. Adjusted* odds ratios and 95% confidence intervals for potential risk factors for SGA among premature infants

Factors	*Odds ratios*	*95% Confidence intervals*
Black race	2.2	1.4, 3.5
Toxemia	3.2	1.7, 6.1
Other hypertension	1.5	0.6, 3.8
Weight gain (reference >15 lb)		
≤5 lb	4.0	1.8, 8.8
>5, ≤10 lb	1.7	0.9, 3.5
>10, ≤15 lb	1.4	0.8, 2.6
Missing	4.9	1.9, 12.6
Prepregnant weight (reference 120 to 159 lb)		
≤119 lb	1.0	0.6, 1.6
≥160 lb	1.5	0.8, 3.0
Missing	0.5	0.2, 1.5
Smoking (reference never)		
Past only	1.0	0.5, 1.9
Current		
½ pack/day	1.2	0.7, 2.3
1 pack/day	0.9	0.5, 1.6
>1 pack/day	1.1	0.5, 2.6
Missing	1.0	0.2, 3.9
Parity (≥1)	1.0	0.6, 1.7
Maternal age (reference 20 to 29)		
≤17 yr	1.1	0.5, 2.2
18-19 yr	1.3	0.7, 2.5
30-34 yr	0.7	0.4, 1.5
>34 yr	1.3	0.5, 3.1
Education		
Low	1.3	0.7, 2.4
Missing	0.5	0.1, 2.4
Unemployed	1.7	1.0, 2.9
Missing	1.7	0.4, 6.6
Multivitamins or iron	0.7	0.4, 1.3
Aspirin	1.2	0.6, 2.2
Acetaminophen	1.1	0.7, 1.7
No spontaneous labor	1.3	0.7, 2.3

*Estimates are adjusted simultaneously for gestational age and gender, plus all of the variables listed in this table.

the proportions were quite similar in the SGA and AGA groups (42% and 43%, respectively).

Although ours is the largest study to date of risk factors for SGA birth within a preterm population, the study is not without its limitations. Because the data from 30% of the women in our study did not contain information about their last menstrual period, (LMP), we estimated the gestational age of infants from Dubowitz infant scores[7] rather than from the LMP. Spinnato et al.[14] have shown that Dubowitz ages are systematic overestimates of (clinically corroborated) LMP derived ages, especially for infants <33 weeks' gestation. However, this careful comparative study found no evidence that Dubowitz ages were differentially inaccurate for SGA versus AGA babies. Thus the consequence of using gestational age estimates derived from Dubowitz scores rather than (clinically corroborated) LMP reports is that the percent of SGA may be overestimated because the use of a cut-off value for a higher Dubowitz gestational age (rather than the lower LMP gestational age) may misclassify as SGA some babies who are really AGA. The effect of this nondifferential misclassification would be to dilute the observed results in the direction of the null. Therefore positive results that emerge in spite of this potential bias (such as the effects of black maternal race, maternal toxemia, and maternal weight gain) may be underestimates of the true effects of those variables. However, for null results (such as the effect of maternal cigarette smoking) the interpretation is more complicated. The observed null result could reflect a true null result, or it could result from the dilution (due to nondifferential misclassification) of a small-to-moderate-sized effect. Neither option can be conclusively excluded on the basis of this study alone.

The effect of maternal smoking on risk of SGA birth among premature infants remains an open question, although the combination of positive and null results from this study suggests that within the already high-risk domain of premature infants, maternal smoking may not confer any added risk for SGA status. However, for maternal toxemia, weight gain, and race, all three are risk factors for prematurity among AGAs, for SGA among term babies,[4, 10, 12] and, as suggested by the present study, risk factors for SGA among premature infants.

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Treatment of unruptured tubal pregnancy by laparoscopic instillation of hyperosmolar glucose solution

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Sixty patients with unruptured tubal pregnancy were treated with local laparoscopic instillation of 50% glucose solution. This treatment was successful in 49 (98%) of 50 patients with an initial serum human chorionic gonadotropin level of ≤2500 mU/ml and in six (60%) of 10 with an initial level >2500 mU/ml. No side effects were seen. The average hospital stay of patients who did not require a second intervention was 5.2 days (range 3 to 10). The average time between glucose instillation and the decline of serum human chorionic gonadotropin levels below the level of detectability was 21.3 (±14.3) and 30.2 (±10.9) days in patients with serum levels ≤2500 mIU/ml and >2500 mIU/ml, respectively. Five patients (8%) underwent a second laparoscopy (n = 4) or laparotomy (n = 1) because of stable or increasing human chorionic gonadotropin levels and progressing clinical symptoms. We conclude that laparoscopic instillation of hyperosmolar glucose solution is safe, technically simple, and effective in the treatment of unruptured tubal pregnancies associated with a serum human chorionic gonadotropin level ≤2500 mIU/ml. (Am J Obstet Gynecol 1992;166:1378-81.)

Key words: Tubal pregnancy, laparoscopy, conservative treatment

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A variety of medical, laparoscopic, and surgical techniques have been developed to preserve the affected tube in those patients with a tubal pregnancy wanting to preserve fertility.[1, 2] Medical efforts in this direction led to the use of methotrexate, which is effective against tumors of the trophoblast.[3] A similar effect was

achieved by prostaglandins.[4] However, both prostaglandins and methotrexate can cause systemic side effects.[5, 6]

Laparoscopic local application of hyperosmolar glucose causes local necrobiosis, and we have used it for the treatment of unruptured tubal pregnancies. A prospective, randomized study showed hyperosmolar glucose to be as effective as prostaglandins in this setting.[7] The current report presents our experience with 60 consecutive patients treated with laparoscopic local instillation of 50% glucose solution into an unruptured tubal pregnancy.

Patients and methods

A total of 127 unruptured tubal pregnancies were treated at our hospital in the 22-month period between February 1, 1989, and December 1, 1990. A diagnosis of tubal pregnancy was made on the basis of history, clinical and pelvic examination, levels of human chorionic gonadotropin (hCG) in the urine and serum, and vaginal ultrasonographic studies. All diagnoses were confirmed by laparoscopy. A total of 77 patients received local medical treatment; 60 of these patients were treated with hyperosmolar glucose solution and are the subject of this report. The remaining 17 patients were treated with prostaglandins according to the protocol of an earlier study.[7] Preoperative criteria for an attempt at treatment with glucose were written: informed consent, desire for preservation of fertility, and stable circulation with no signs of hemorrhagic shock. Intraoperative inclusion criteria were complete visualization of the small pelvis, an intact serosal covering of the tube, ≤200 ml of hematoperitoneum, and no active bleeding.

After inspection of the pelvic organs, any blood present was suctioned out of the abdomen. The affected tube was grasped with atraumatic forceps introduced through a second, suprasymphyseal incision. Five to 20 milliliters of a 50% glucose solution was injected into the pregnancy through a 15-gauge needle 40 cm long introduced through the second suprasymphyseal incision. Injection was stopped when there was notable swelling of the tube or when glucose solution was seen escaping from the fimbrial end of the tube. Any residual fluid was suctioned out of the pouch of Douglas.

Pulse, blood pressure, and red blood cell counts were monitored for the first 48 hours. hCG levels in the urine and serum were measured at least once preoperatively and daily during the hospital stay. The overall correlation between hCG levels in the urine and the serum was 0.8 ($p < 0.0001$, Spearman's rank correlation test). The patients were discharged from the hospital when hCG levels declined for 2 subsequent days. Urinary hCG levels were measured every 2 to 3 days until they declined below 50 mIU/ml, the lower limit of detection (Test Pack HCG urine, Organon Teknika, Boxtel, Netherlands). Thereafter, hCG levels were measured in the serum (hCG MAIA clone immunoradiometric assay, magnetic solid phase; Serono Diagnostics, Norwell, Mass.) at 1-week intervals until the lower limit of detection (5 mIU/ml) was reached. All patients were offered hysterosalpingography 3 months or more after the procedure.

Table I. Clinical data according to initial serum hCG concentration of 60 patients undergoing laparoscopic instillation of hyperosmolar glucose solution into unruptured tubal pregnancy (mean ± SD)

	Initial serum hCG	
	≤2500 mIU/ml	*>2500 mIU/ml*
Age (yr)	26.8 ± 4.8	27.4 ± 5.0
Gestational age (days)	49.4 ± 8.3	47.2 ± 7.3
Urinary hCG (mIU/ml)	811 ± 758	4100 ± 2644
Serum hCG (mIU/ml)	582 ± 533	4348 ± 2007
Size of pregnancy (cm)	3.0 × 1.5	3.3 × 1.8
Hemoperitoneum (ml)	20 ± 31	22 ± 29
Localization (right)	56	40
Primigravid women (%)	34	50

Results

The clinical data of the patients are shown in Table I. Fifty-seven of the tubal pregnancies were located in the ampullary or isthmoampullary portion of the tube; three were located in the tubal isthmus. The average hospital stay of the 55 patients who did not require a second operation was 5.2 days (range 3 to 10). The patients were separated into two groups on the basis of preoperative serum hCG levels of ≤2500 mIU/ml ($n = 50$) and >2500 mIU/ml ($n = 10$). There were no differences in the results according to the amount of glucose injected.

Tubal pregnancies with initial serum hCG ≤2500 mIU/ml. The courses of serum hCG levels in these 50 patients are shown in Fig. 1. Glucose instillation was successful in 49 (98%) patients. The average time between glucose instillation and the decline of serum hCG below the level of detectability was 21.3 (± 14.3) days. One patient with an initial serum hCG level of 1200 mIU/ml undewent a second laparoscopy 28 days after the first procedure because of a renewed increase of hCG levels and lower abdominal pain. The pregnancy had not yet ruptured but was larger than at primary surgery; the affected tube was removed endoscopically at the patient's request.

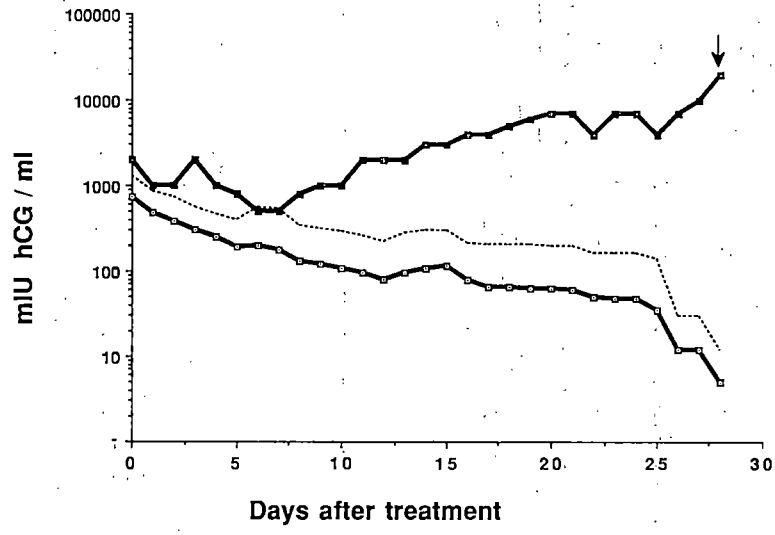


Fig. 1. Mean course of hCG excretion in 50 patients with initial serum hCG level ≤2500 mIU/ml (*dashed line,* SD). *Lower solid line* represents course of 49 patients treated successfully and *upper solid line* shows course of patient who required reintervention *(arrow).*

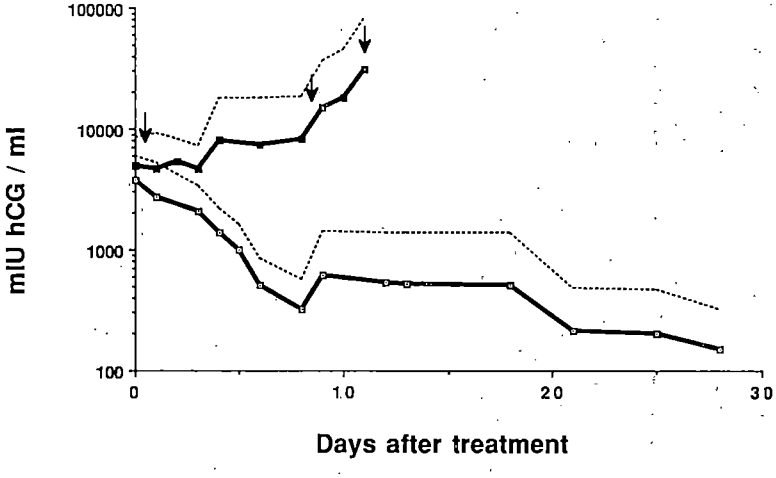


Fig. 2. Mean course of hCG excretion in 10 patients with initial serum hCG level >2500 mIU/ml (*dashed lines,* SD). *Upper solid line* indicates course in three patients who required reintervention *(arrows). Lower solid line* indicates course in six patients treated successfully. Patient who underwent laparotomy for ruptured tubal pregnancy on day 32 did not undergo follow-up examinations.

Tubal pregnancies with initial serum hCG >2500 mIU/ml. The courses of the serum hCG levels in these 10 patients are shown in Fig. 2. Local glucose instillation was successful in six (60%) patients. The average time between glucose instillation and the decline of serum hCG below the level of detectability was 30.2 (± 10.9) days. Four patients underwent a second operation at 1, 9, 11, and 32 days, respectively. One of these patients had an isthmic tubal pregnancy. Three patients underwent a second laparoscopy with salpingectomy (n = 2) or salpingotomy (n = 1). One patient did not come to follow-up examinations and underwent laparotomy for a ruptured tubal pregnancy at another hospital 32 days after glucose instillation.

Follow-up. Twenty-two patients underwent hysterosalpingography 3 to 6 months after glucose instillation. Patency and normal configuration of the affected tube were seen in 18 patients; four patients showed occlusion of the affected tube. Twelve patients achieved an intrauterine pregnancy. Two of these patients probably became pregnant through the affected tube because hysterosalpingography had shown occlusion of the contralateral tube. Two patients developed a renewed tubal pregnancy (one in the contralateral and one in the same tube).

Comment

Highly sensitive and specific hCG assays and vaginal ultrasonography have made possible the early diagnosis and treatment of tubal pregnancies before rupture.[8, 9]

The higher the hCG level, the greater the mass of viable trophoblastic tissue and the greater the risk of rupture.[10] Pansky et al.[11] have recently reviewed nonsurgical modalities for the management of tubal pregnancy.

Local application of methotrexate or prostaglandins has been successful in about 80% to 90% of unruptured tubal pregnancies.[12-14] However, both substances can cause systemic side effects, and the application of methotrexate in the immediate vicinity of the ovary seems at least questionable. Hyperosmolar glucose causes local dehydration and necrobiosis of the trophoblastic tissue and thus resolution of the tubal pregnancy. A second laparoscopy of isolated patients has shown no signs of damage to the tubal wall. None of the patients in the current series had side effects attributable to glucose instillation.

The average time required for serum hCG levels to decline below the level of detectability (21.3 ± 14.3 days in patients with an initial level of ≤2500 mIU/ml) is of concern insofar as the patients must be followed during this period. The decline in hCG levels is obviously more precipitous after interventions such as salpingotomy or salpingectomy that remove the trophoblast. However, in a study comparing glucose instillation with prostaglandin treatment there were no differences in the resolution time of hCG levels.[7] In a series of 12 patients treated by transvaginal intratubal methotrexate administration, Tulandi et al.[15] reported a resolution time of 14 to 120 days from injection to undetectable hCG levels. However, the initial serum hCG levels in these patients ranged from 1593 to 21,000 mIU/ml. The mean resolution time in a series of 14 patients with initial hCG levels of 78 to 8000 mIU/ml given only expectant treatment was 20.3 ± 13.1 days.[16] Overall, it is difficult to compare the hCG resolution times among different studies because of different inclusion criteria, particularly initial hCG levels.

The long average hospital stay of the successfully treated patients in this series (5.2 days) was mainly due to the design of the research protocol. Also, the health care system in Austria reimburses hospitals for the number of days a patient is in the hospital and patients thus tend to be hospitalized longer than necessary. We now discharge clinically stable patients on the day after surgery if the hCG is not rising and follow them on an outpatient basis.

The tubal mucosa is not a favorable site for nidation, and tubal pregnancies can heal spontaneously.[11] In the largest study of initial expectant management, Fernandez et al.[16] found nine (64%) of 14 ampullary tubal pregnancies to resolve spontaneously. Seven of these nine patients had initial hCG levels ≤1000 mIU/ml, as did 43 patients in our series. However, at present it is difficult to distinguish between pregnancies that will resolve spontaneously and those that will rupture the tube.[11]

The results of this series suggest that local instillation of hyperosmolar glucose is safe, effective and free of side effects in the treatment of unruptured tubal gestations with a serum hCG level ≤2500 mIU/ml. The technique is simple and the integrity of the tubal wall is preserved. Glucose instillation does not seem indicated for ectopic gestations associated with a serum hCG level >2500 mIU/ml.

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The relationship between acute inflammatory lesions of the preterm placenta and amniotic fluid microbiology

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OBJECTIVE: Our objective was to determine the relationship between microbial invasion of the amniotic cavity and the presence and severity of acute inflammatory lesions in the placenta.

STUDY DESIGN: Placental histologic and amniotic fluid microbiologic studies were performed in 92 consecutive patients who were admitted with preterm labor and intact membranes and delivered within 48 hours after amniocentesis.

RESULTS: The prevalence of a positive amniotic fluid culture was 38% (35 of 92). There was a strong association between the presence and severity of inflammation in the amnion, chorion-decidua, umbilical cord, and chorionic plate and the results of the amniotic fluid culture ($p < 0.0001$ for each tissue section). Three patterns of inflammation of the chorion-decidua were identified: marginating, nonmarginating, and a mixed pattern. The marginating and the mixed patterns of inflammation were strongly associated with the presence of a positive amniotic fluid culture. Acute inflammation of the chorionic plate was the most sensitive indicator of a microbial invasion of the amniotic cavity (sensitivity 96.6%), and funisitis and umbilical vasculitis had the highest specificity (85.7%).

CONCLUSION: The presence of acute inflammatory lesions of the chorioamniotic membranes can serve as a marker of microbial invasion of the amniotic cavity. (Am J Obstet Gynecol 1992;166:1382-8.)

Key words: Chorioamnionitis, intraamniotic infection, placenta, preterm labor, prematurity, parturition, inflammation

Acute inflammation of the chorioamniotic membranes is one of the most common histopathologic lesions of the placenta. Traditionally, histologic chorioamnionitis has been considered an indicator of amniotic fluid infection.[1-8] However, there is a paucity of scientific evidence to support this view. Previous studies have demonstrated an association between acute inflammatory lesions of the placenta and the recovery of microorganisms from the subchorionic plate[9,10] and the chorioamniotic space.[11] However, recovery of microorganisms from these extraamniotic sites cannot be used as proof of an amniotic fluid infection. It seems logical that the evidence supporting a relationship between histologic chorioamnionitis and amniotic fluid infection can only be derived from a comparison of pathologic placental examination and amniotic fluid microbiologic studies. Only one study has examined the relationship between amniotic fluid microbiologic features and placental pathology; this study used transcervical collection of amniotic fluid for microbiologic studies and therefore is limited in its ability to discriminate between specimen contamination and the true microbiologic state of the amniotic cavity.[12] Another limitation of this study is that patients had their membranes ruptured, which could lead to intrapartum microbial invasion of the amniotic cavity and cloud interpretation of the data.

The purpose of this study was to determine the relationship between the presence, severity, distribution, and pattern of acute inflammatory lesions of the placenta and microbiologic findings of the amniotic fluid. To study this issue we have used a unique data set: microbiologic studies of amniotic fluid and placentas obtained from women with preterm labor and intact membranes who were delivered within 48 hours of amniocentesis.

Material and methods

The placental histologic types and amniotic fluid microbiologic features were examined in consecutive patients admitted to Yale–New Haven Hospital who met

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Table I. Grading system for acute intrauterine inflammations

Amnion and chorion-decidua
Grade 1: One focus of at least five polymorphonuclear leukocytes
Grade 2: More than one focus of grade 1 inflammation or at least one focus of 5 to 20 polymorphonuclear leukocytes
Grade 3: Multiple or confluent foci of grade 2
Grade 4: Diffuse and dense acute inflammation
Umbilical cord
Grade 1: Polymorphonuclear leukocytes within inner third of umbilical vein wall
Grade 2: Polymorphonuclear leukocytes within inner third of at least two umbilical vessel walls
Grade 3: Polymorphonuclear leukocytes in perivascular Wharton jelly
Grade 4: Panvasculitis and funisitis extending deep into Wharton jelly
Chorionic plate
Grade 1: One focus of at least five polymorphonuclear leukocytes in subchorionic fibrin
Grade 2: Multiple foci of grade 1 in subchorionic fibrin
Grade 3: Few polymorphonuclear leukocytes in connective tissue or chorionic plate
Grade 4: Numerous polymorphonuclear leukocytes in chorionic plate and chorionic vasculitis

Modified from Salafia et al.[14]

these criteria: (1) preterm labor with intact membranes (gestational ages 22 to 36 weeks) and (2) preterm delivery within 48 hours after amniocentesis. Data from some of these patients have been reported in other studies by our group; data from placental histologic examination have not been previously reported. The standard of care at our institution is to offer amniocentesis for microbiologic assessment of the amniotic cavity to all women admitted with the diagnosis of preterm labor with intact membranes. Patients gave written informed consent for amniocentesis. Additionally, some patients gave permission to withdraw additional fluid for research purposes. Women with preterm premature rupture of membranes were excluded from this study because microbial invasion of the amniotic cavity in the context of rupture of membranes may be a fundamentally different pathologic process to that occurring in preterm labor with intact membranes.

After amniocentesis, amniotic fluid was transported immediately to the laboratory in a capped plastic syringe. These conditions precluded air contact with the specimen. Plating occurred within 30 minutes after collection in most cases. Microbiologic studies included amniotic fluid cultures for aerobic and anaerobic bacteria and *Mycoplasma hominis* and *Ureaplasma urealyticum*, by methods previously described in detail.[13] Placental microbiologic studies, including cultures of the subchorionic fibrin and chorioamniotic space, were not performed. This is not the standard of practice in our institution. The relationship between cultures from these sites and placental histologic type has been addressed by other authors.[9-11]

Gross and microscopic studies of the placentas were performed according to an established protocol previously described by Salafia et al.[14] This protocol has been used for the study of the prevalence and distribution of acute placental inflammatory lesions in uncomplicated term pregnancies.[14] Briefly, tissue samples included sections of umbilical cord, the chorionic plate (taken from an area with minimal subchorionic fibrin), and a roll of membranes extending from the area of membrane rupture to the placental margin. All blocks of tissue were fixed with 10% buffered formalin and embedded in paraffin. Sections of tissue blocks were stained with hematoxylin and eosin and read by a perinatal pathologist (C.M.S.) who was blinded to the clinical information. The presence of acute inflammation of the extraplacental membranes, cord, and chorionic plate was noted and graded with a semiquantitative scale (Table I). Any tissue that contained cells identified as polymorphonuclear leukocytes was graded as positive for acute inflammation.

The pattern of inflammation in the chorion-decidua was classified as marginating, nonmarginating, or mixed, a combination of both. The marginating pattern consisted of the presence of neutrophils piled up along the interface between the amnion and chorion-decidua (Fig. 1). A nonmarginating pattern was defined as any other distribution of neutrophils in the chorion-decidua. Frequently this pattern appears as a destructive decidual inflammatory process (necrotizing type of inflammation) (Fig. 2).[14]

Contingency tables were generated with each type of histologic section (umbilical cord, chorionic plate, amnion, chorion, and pattern of inflammation) and amniotic fluid culture results. A χ^2 for trend was used to analyze contingency tables, which included ordered data. The data were analyzed with the True Epistat statistical package (Epistat Services, Richardson, Tex.). A replication study was conducted for the various tissue sections, and the intraclass correlation coefficients and the 95% confidence intervals were calculated for each one.[15] Histologic slides from 20 patients were read by



Fig. 1. Acute marginating chorioamnionitis-deciduitis, grade 3 *(thin arrow)*, and amnionitis, grade 2 *(thick arrow)*. (Hematoxylin and eosin. Original magnification × 10.)

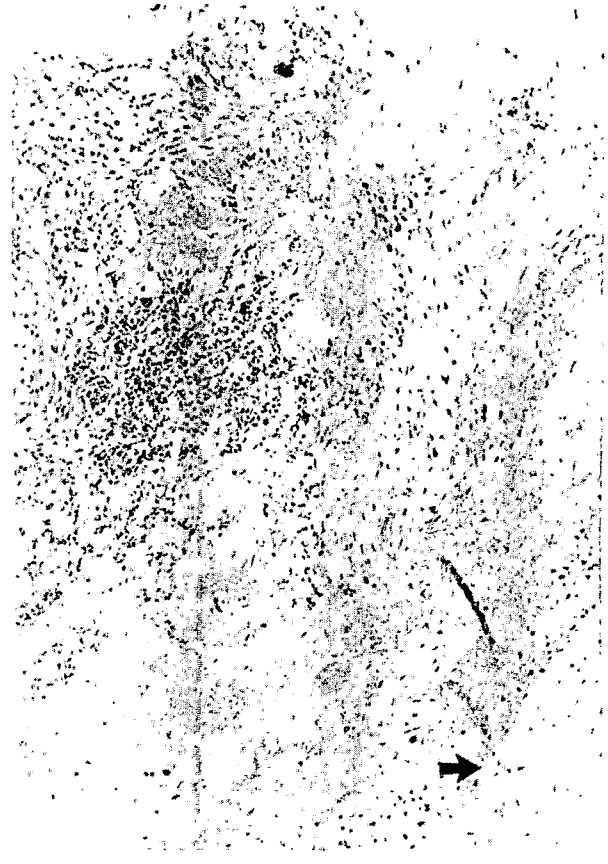


Fig. 2. Acute nonmarginating (necrotizing) deciduitis, grade 1, with maternal neutrophils focally present in decidua (*arrow* at junction of cellular and connective tissue chorion). (Hematoxylin and eosin. Original magnification × 10.)

a pathologist (C.M.S.) on 10 different occasions. The observer was kept blinded from clinical information and previous readings.

Results

This study included 92 patients who met the entry criteria. Positive amniotic fluid cultures were detected in 38% (35 of 92) of the patients. Table II illustrates the amniotic fluid microbial isolates from women in this study. No correlation could be demonstrated between the inoculum size and the severity of inflammation in any histopathologic sections. Table III illustrates the distribution of acute inflammatory lesions in the membranes, chorionic plate, and umbilical cord according to the result of the amniotic fluid culture. In each instance the more severe the inflammatory process, the more likely it was that the patient had a positive amniotic fluid culture.

There was a correlation between the grade of inflammation in the amnion and that of the chorion-decidua ($r = 0.46$; $p = 0.00004$, Spearman rank test). A similar correlation was found between the grade of inflammation of the umbilical cord and in the chorion-decidua ($r = 0.30$; $p = 0.01$, Spearman rank test). We also found a strong correlation between the presence or absence of inflammation in the chorionic plate and inflammation in other tissue sections ($p < 0.01$). However, it was not possible to demonstrate a correlation between the grade of inflammation in the chorionic plate and that observed in any other tissue sections ($p > 0.05$).

Table IVA displays the frequency and distribution of the pattern of inflammation in the chorioamniotic membranes according to the results of the amniotic fluid culture. Table IVB displays the results of multiple comparisons with partition χ^2. The presence of any inflammation (nonmarginating, marginating, or mixed) was significantly associated with a positive amniotic fluid culture. Furthermore, inflammation with a marginating pattern (alone or with a mixed pattern) was also associated with a positive amniotic fluid culture when compared with a nonmarginating pattern alone. Additionally, inflammation in the chorion-decidua of the mixed type was significantly associated with a

positive amniotic fluid culture compared with a nonmarginating or marginating pattern alone. Finally, inflammation in the marginating pattern alone was significantly associated with a positive culture when compared with inflammation in only the nonmarginating pattern.

Table V shows the conventional statistics used to describe a screening test, which placental examination could be with respect to microbial invasion of the amniotic cavity. In spite of being based on a relatively small number, the high sensitivity of the chorionic plate and the high specificity of the umbilical cord are clearly evident and worth noting. The positive predictive value indicates that about 66% of those defined as being infected would actually be so, but 96.6% (negative predictive value) of those defined as not being infected would truly not be, given the same prevalence of disease found in our study population.

A replication reliability study was conducted on the amnion, chorion-decidua, chorionic plate, and umbilical cord. The intraclass correlation coefficients and the one-sided 95% confidence intervals are presented in Table VI. The values indicate excellent agreement for readings from umbilical cord and amnion. The agreements for the chorionic plate and chorion-decidua were also very good.

Table II. Microbial isolates from patients with positive amniotic fluid cultures

Microorganisms	*Incidence*
Mixed infection*	7
Fusobacterium sp.	6
Gardnerella vaginalis	6
Mycoplasma hominis	5
Ureaplasma urealyticum	4
Peptostreptococcus	3
Bacteroides sp.	3
Lactobacillus sp.	2
Streptococcus viridans	1
Streptococcus agalactiae	1
Haemophilus influenzae	1
Veillonella parvula	1
Staphylococcus aureus	1
Capnocytophaga	1

*More than three microorganisms isolated from a patient.

Comment

The results of this study clearly demonstrate a strong association between the presence, severity, and distribution of acute inflammatory lesions in the placenta and the recovery of microorganisms from the amniotic fluid. These findings support the traditional view that there is a relationship between the presence of acute inflammatory lesions of the placenta and membranes and intraamniotic infection. A similar relationship has been documented in the context of term gestation with a different method for amniotic fluid microbiologic studies (transcervical aspiration).[12]

The prevalence of positive amniotic fluid cultures in this study was 38%, which is higher than the 21.6% that we previously reported in a large prospective study.[16] The apparent discrepancy is most likely related to one of the enrollment criterion of this study: delivery within 48 hours of amniocentesis. This criterion was chosen to restrict the time interval between amniocentesis and delivery, so that the microbiologic status of the amniotic cavity and histologic examination of the placenta could be compared in a temporally meaningful way. In previous studies we have documented that women with positive amniotic fluid cultures have a shorter interval between amniocentesis and delivery than women with negative cultures.[16]

We examined the frequency and degree of acute inflammatory lesions in several tissue sections, including the umbilical cord, chorionic plate, amnion, and chorion-decidua. These tissue sections afford the possibility of examining both fetal and maternal responses to the presence of an intraamniotic infection. Although funisitis, umbilical vasculitis, and chorionic vasculitis are fetal responses,[17, 18] neutrophil infiltration of the chorionic plate, amnion, chorion, and decidua are essentially the consequence of a maternal response to infection.[18]

Our data showed a correlation between the presence and degree of inflammation in the amnion and chorion-decidua tissue sections. Because neutrophils in the avascular amnion and chorion-decidua are of maternal origin and these tissues are immediately adjacent to each other, it is not surprising to find a high degree of correlation between the severity of inflammation in these tissue sections. Similar observations have been reported in studies of placentas of women at term.[12]

In spite of the strong association between the presence or absence of inflammation in the chorionic plate and inflammation in other tissues sections, we could not demonstrate a correlation between the grade of inflammation in the chorionic plate and that observed in any other tissue sections. The degree of inflammation in the chorionic plate was often more severe than the one observed in other tissue sections. The higher sensitivity of the chorionic plate in the detection of a positive amniotic fluid culture can be attributed to the greater blood flow and availability of neutrophils in the intervillous space (in direct proximity to the chorionic plate) than that in the decidua capsularis.

Although inflammation of the umbilical cord was the least sensitive indicator of a positive amniotic fluid culture, it had the highest positive predictive value. The lower sensitivity is easily explained if one takes into account the physical distance between the chemotactic

Table III. Presence and severity of inflammation in different tissue sections according to amniotic fluid culture results

Tissue	Culture	Grade of inflammation					χ^{2*}	df	p
		0	1	2	3	4			
Amnion	Negative	40	8	7	1	0	29.481	1	<0.0001
	Positive	6	8	7	5	8			
Chorion-decidua	Negative	25	8	10	11	1	25.666	1	<0.0001
	Positive	2	8	3	14	10			
Umbilical cord	Negative	42	8	1	3	1	25.319	1	<0.0001
	Positive	9	6	5	6	9			
Chorionic plate	Negative	22	7	4	3	8	30.596	1	<0.0001
	Positive	1	0	2	5	22			

*All comparisons were performed with χ^2 for trends.

Table IVA. Pattern of inflammation in chorion-decidua

Culture	No inflammation		Pattern of acute inflammation						χ^2	p
			Necrotizing		Marginating		Mixed			
	Observed	Expected	Observed	Expected	Observed	Expected	Observed	Expected		
Negative	23	17.2	12	8.6	8	10.4	6	12.9	19.57	<0.0002
Positive	5	10.8	2	5.4	9	6.6	15	8.1		

Table IVB. Partition of χ^2

Comparisons	df	χ^2	p
Overall	3	19.57*	0.000
No inflammation vs any inflammation	1	7.92	0.005
Nonmarginating vs marginating vs mixed	2	11.06*	0.004
Nonmarginating or marginating vs mixed	1	6.47	0.011
Nonmarginating vs marginating	1	5.01	0.025

*The χ^2 values do not sum exactly because of rounding.

stimuli of bacteria in the amniotic fluid and the fetal white blood cells contained within the vessels of the umbilical cord. Temporally, umbilical vasculitis and funisitis are probably late events in the course of an intraamniotic infection. Therefore these inflammatory lesions would be expected to have a high positive predictive value for the detection of intraamniotic infection. This expectation was met by our data. Our observations are consistent with those of Maudsley et al.,[19] who demonstrated that the more advanced the inflammation in the umbilical cord section, the more likely that other tissue sections had inflammation.

A new observation made in this study is that a marginating pattern of inflammation in the chorion-decidua was strongly associated with a positive amniotic fluid culture. The distribution of neutrophils suggests that the source of chemotactic stimuli is located in the amniotic fluid. In contrast, the distribution of neutrophils in the nonmarginating necrotizing inflammatory lesion suggests that the location of the chemotactic stimuli is in the decidua.

The pathways of ascending intrauterine infection have not been clearly elucidated. One possibility is that microorganisms from the vagina and cervix transverse the membranes adjacent to the cervix and colonize the amniotic fluid. Alternatively, microorganisms may ascend to broadly colonize the chorioamniotic membranes and decidua and only at a later stage gain access to the amniotic fluid.

We propose that these two pathways of infection should lead to different histologic patterns of inflammation. In the first pathway, a predominant marginating pattern of inflammation would be found, because the chemotactic stimuli would be generated in the amniotic cavity. This tenet is consistent with the results of this study in which a marginating pattern was signifi-

Table V. Diagnostic indices of different tissue sections for the identification of a positive amniotic fluid culture*

	Sensitivity	*Specificity*	*Positive predictive value*	*Negative predictive value*
Amnion	82.3 (0.65, 0.93)	78.4 (0.64, 0.88)	71.1 (0.55, 0.85)	86.9 (0.73, 0.95)
Chorion-decidua	87.0 (0.70, 0.96)	57.6 (0.43, 0.71)	55.1 (0.40, 0.69)	88.2 (0.75, 0.96)
Chorionic plate	96.6 (0.82, 0.99)	65.9 (0.50, 0.79)	65.9 (0.50, 0.79)	96.6 (0.82, 0.99)
Umbilical cord	74.2 (0.56, 0.87)	85.7 (0.72, 0.94)	78.7 (0.61, 0.91)	82.3 (0.69, 0.91)
Pattern	77.4 (0.58, 0.90)	71.4 (0.56, 0.83)	63.1 (0.46, 0.78)	83.3 (0.68, 0.93)

95% Confidence limits are shown in parentheses.

*Diagnostic indices were calculated with the following criteria for the definition of acute inflammation in various tissue sections: amnion, grades 1 through 4; chorion-decidua, grades 2 through 4; umbilical cord, grades 1 through 4; chorionic plate, grades 2 through 4.

cantly associated with the presence of positive amniotic fluid culture. In the second pathway, microbial invasion of the decidua would lead to a nonmarginating necrotizing pattern. We believe that this type of inflammatory lesion results from the appearance of neutrophils in the decidua where the foci of bacteria are located. Necrotic lesions would be the consequence of local tissue destruction by the microorganisms. If this pathway is used, the infection would be restricted mainly to the decidua and chorion, and the prevalence of positive amniotic fluid cultures should be low. Our data support this interpretation. Indeed, the prevalence of positive amniotic fluid cultures was similar in patients without inflammatory lesions of the placenta and in patients with a nonmarginating pattern. Finally, if microorganisms that initially cause an extraamniotic infection (chorionitis-deciduitis) invade the amniotic cavity or if microorganisms traverse intact membranes and colonize the decidua, a mixed pattern of inflammation would be observed. Under these circumstances, the chemotactic stimuli reside in both the decidua and amniotic cavity.

A major finding in this study is that acute inflammatory lesions of the placenta can be present in the absence of positive amniotic fluid cultures for microorganisms. Several explanations can be invoked: (1) The infection is located extraamniotically; (2) the inoculum size required to elicit an inflammatory response may be smaller than that which is required for isolation of microorganisms by standard microbiologic techniques; (3) nonviable microorganisms may release chemotactic factors (i.e., endotoxin) that elicit inflammation but would not yield a positive amniotic fluid culture; (4) inflammation may be caused by microorganisms that escape detection by the microbiologic techniques used in this study; (5) infection may not be the only cause of acute inflammatory lesions; (6) an intraamniotic infection may have developed in the interval between amniocentesis and delivery.

In conclusion, the results of the present study support the view that in the setting of preterm labor with intact membranes, the majority of inflammatory lesions of the chorioamniotic membranes are associated with microbial invasion of the amniotic cavity in the absence of overt clinical signs of infection. Placental pathologic examination provides a noninvasive means of assessing the likelihood of microbial invasion of the amniotic cavity.

Table VI. Intraclass correlation coefficient and one-sided 95% confidence intervals for the various tissue sections

Tissue	*Correlation coefficient*	*One-sided 95% confidence interval*
Amnion	0.9317	0.8917
Chorion-decidua	0.8335	0.7479
Chorionic plate	0.8869	0.8251
Umbilical cord	0.9686	0.9494
Pattern	0.7090	0.5830

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Maternal serum α-fetoprotein, β-human chorionic gonadotropin, and unconjugated estriol levels in midtrimester trisomy 18 pregnancies

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OBJECTIVE: The purpose was to evaluate the levels of maternal serum human chorionic gonadotropin, α-fetoprotein, and unconjugated estriol in trisomy 18 pregnancies compared with normal singleton pregnancies.

STUDY DESIGN: Sera from 14 trisomy 18 pregnancies (13 retrospectively and one prospectively ascertained) were analyzed for human chorionic gonadotropin, α-fetoprotein, and unconjugated estriol.

RESULTS: The α-fetoprotein levels in the 10 trisomy 18 pregnancies without open neural tube or ventral wall defect had a median of 0.65 multiple of the median, although two had α-fetoprotein levels above 2.5 multiples of the median. The human chorionic gonadotropin levels had a median of 0.32 multiple of the median and the unconjugated estriol levels had a median of 0.56 multiple of the median. Although most women with trisomy 18 pregnancies had serum human chorionic gonadotropin levels that were <1.0 multiple of the median, three had markedly elevated human chorionic gonadotropin levels (>5.0 multiples of the median).

CONCLUSION: Our data are partially consistent with those previously published but suggest the possibility of a bimodal distribution of α-fetoprotein and human chorionic gonadotropin levels in trisomy 18–affected pregnancies, unrelated to a neural tube or abdominal wall defect. The efficiency of screening for trisomy 18 prospectively, using the three serum markers, requires further evaluation. (AM J OBSTET GYNECOL 1992;166:1388-92.)

Key words: α-fetoprotein, human chorionic gonadotropin, estriol, trisomy 18, prenatal screening

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An association between low levels of maternal serum α-fetoprotein (AFP) and both fetal trisomy 21 and trisomy 18 was noted by Merkatz et al.[1] An association between low maternal serum levels of unconjugated estriol in midtrimester and fetal trisomy 21 was found by Canick et al.[2] Bogart et al.[3, 4] also discovered an association of elevated maternal serum β-human chori-

Table I. Data on 14 trisomy 18 pregnancies

Case No.	Multiples of the median			Maternal age (yr)	Gestational age (wk)
	Maternal serum AFP	β-hCG	Unconjugated estriol		
1	0.39	0.25	0.65	28	18.3
2	0.50	1.30	0.56	29	15.9
3	3.16	8.45	0.67	29	16.0
4	0.26	0.30	0.40	25	16.0
5	1.82	0.13	0.53	28	16.0
6*	0.65	0.26	0.46	23	15.0
7	0.95	0.76	0.48	29	18.0
8	0.65	0.32	0.17	32	17.0
9	4.59	0.16	1.10	26	16.0
10*	4.85	0.24	0.73	38	16.3
11	0.65	15.8	0.74	33	16.0
12	0.72	10.5	0.77	36	16.0
13†	3.26	0.27	1.19	25	15.7
14*	0.58	0.21	0.43	40	16.0
Mean	1.64	2.78	0.65	30	16.3
Median	0.95	0.27	0.63		

*Omphalocele.
†Open spina bifida.

onic gonadotropin (β-hCG) levels with fetal trisomy 21 but low β-hCG levels with fetal trisomy 18. The use of the three serum markers has been suggested as a means of prenatal screening for fetal trisomy 21, inasmuch as the levels of the three markers and maternal age can be used to calculate an estimated risk of trisomy 21.[5]

Canick et al. recently reported data on the levels of β-hCG, unconjugated estriol, and α-fetoprotein in pregnancies with trisomy 18.[6] In 10 pregnancies with trisomy 18, they found low AFP levels in association with very low levels of β-hCG (median 0.27 multiple of the median, range 0.13 to 1.07) and very low levels of unconjugated estriol (median 0.49 multiple of the median, range <0.15 to 0.72). We analyzed serum β-hCG, AFP, and unconjugated estriol levels in 14 trisomy 18 pregnancies (13 retrospectively and 1 prospectively) from our Prenatal Screening Program and compared it with serum from normal, singleton control pregnancies.

Material and methods

Specimens. The Baylor Maternal Serum α-Fetoprotein Screening Program, a medical school–based, not-for-profit prenatal screening program was established in 1981. Women screened by the program come mainly from the Houston area, but are also from other areas of Texas and western Louisiana and include women from both private obstetric practices and public health clinics. The use of low maternal serum AFP levels to screen for increased risk of fetal Down syndrome began in 1985, and over 50,000 women have been screened. Prospective screening using AFP, β-hCG, and unconjugated estriol was initiated in January 1990. Of these, 13 singleton trisomy 18 pregnancies have been ascertained since 1985. Five had low maternal serum AFP levels (Down syndrome risk at delivery ≥1:360) and four had elevated maternal serum AFP levels (>2.5 multiples of the median). These nine women were offered amniocentesis and were confirmed to have trisomy 18 prenatally. In the remaining four pregnancies the women had "normal" maternal serum AFP levels but were delivered of a trisomy 18 infant. One trisomy 18 pregnancy was detected prospectively with maternal serum AFP, β-hCG, and unconjugated estriol among over 3000 women screened in 1990 (patient 14, Table I). These 14 singleton trisomy 18 pregnancies constituted the case group. The control group consisted of 285 randomly selected, previously screened women with known normal pregnancy outcomes. Demographic, pregnancy-specific, and AFP data were retrieved from the computer files and were used without further testing or modification. Gestational age had been based primarily on a woman's last menstrual period unless the gestational age calculated by ultrasonographic biparietal diameter was discrepant by >1 week. Case and control sera that were previously stored at −20° C were retrieved and analyzed for β-hCG and unconjugated estriol.

Laboratory methods. AFP was measured by radioimmunoassay with reagents from Clinical Assays. The sensitivity of the assay was increased by modifying the procedure to a two-step reaction and using a standard curve from 1.2 ng/ml to 60.5 ng/ml. The intraassay and interassay coefficient of variation at this low level was <5%. The AFP concentrations were converted to multiples of the normal median for gestational age on the basis of our own previous data and adjusted for maternal weight using previously published formulas.[7]

β-hCG was determined by a solid-phase, two-site im-

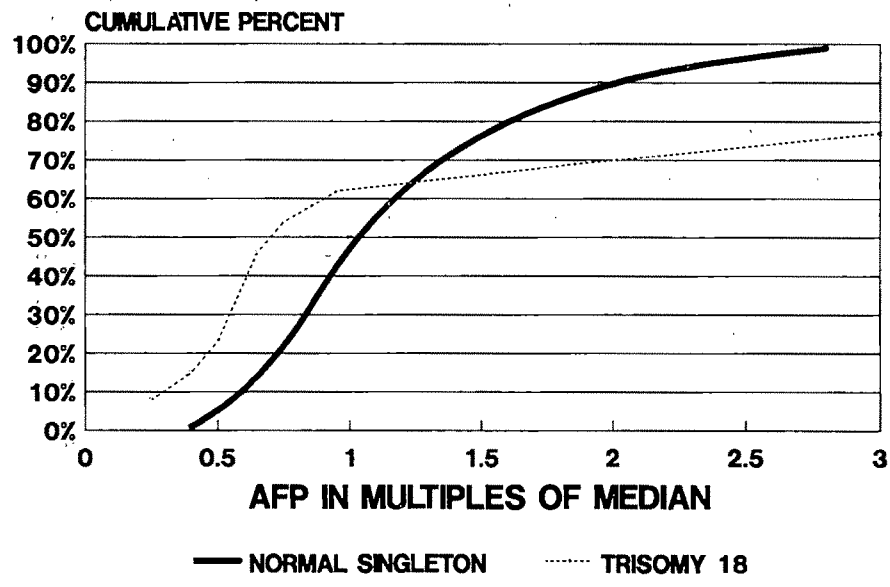


Fig. 1. Cumulative distribution maternal serum AFP levels of 13 trisomy 18 pregnancies and control singleton pregnancies by weight-adjusted multiple of the normal median.

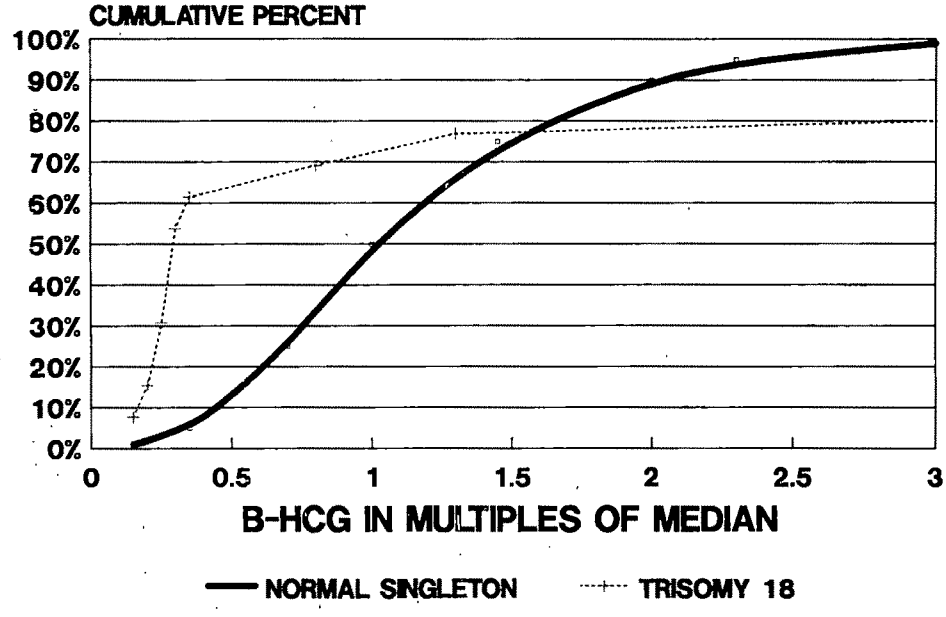


Fig. 2. Cumulative distribution β-hCG levels of 13 trisomy 18 pregnancies and control singleton pregnancies by weight-adjusted multiple of the normal median.

munoenzymatic assay (Tandem-E-HCG Immunoenzymatic assay kit, Hybritech, Inc.). Absorbance was recorded at 405 nm by means of a Photon-Immunoassay analyzer. Sera were diluted 1:500 for testing. Human sera containing β-hCG at 0 mIU/ml and 200 mIU/ml were used as standards.

Unconjugated estriol was determined by a solid-phase radioimmunoassay (Diagnostic Products Corp.). The procedure was modified to detect lower levels of unconjugated estriol at gestational ages <18 weeks. The concentrations of standards and controls ranged from 0.2 to 10 ng/ml. Iodine 125–labeled unconjugated estriol was reduced from 1.0 ml to 925 μl to maintain reaction volume, and controls were used at 1.0 and 5.0 ng/ml.

Results

The data from the 14 trisomy 18 cases are summarized in Table I. The mean gestational age was 16.3 weeks for cases and 17.2 weeks for controls. The mean

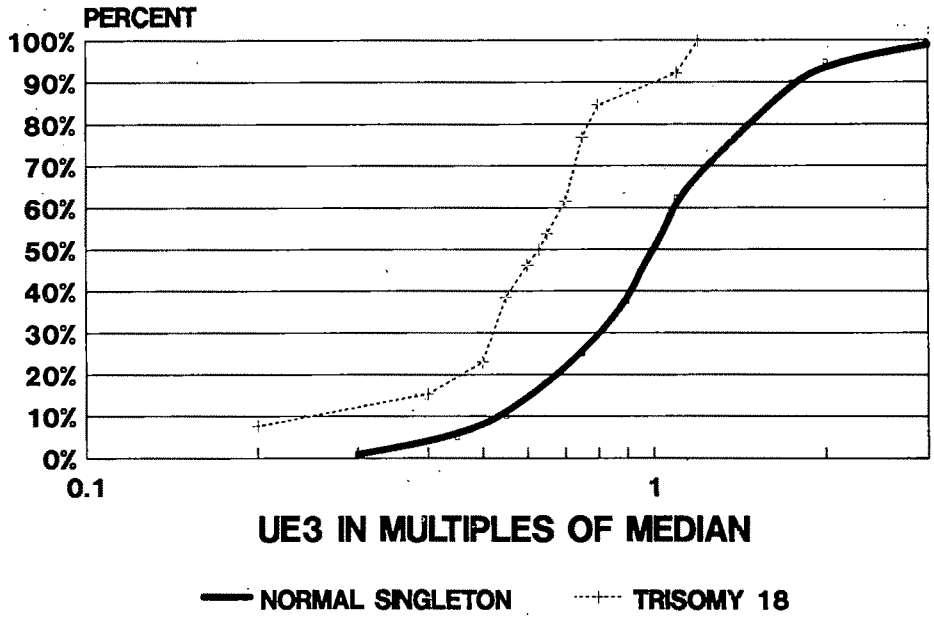


Fig. 3. Cumulative distribution unconjugated estriol levels of 13 trisomy 18 pregnancies and control singleton pregnancies by weight-adjusted multiple of the normal median.

maternal age for cases was 30 years, whereas the control mean was 28 years. The mean weight-adjusted AFP level for all controls was 1.05 multiples of the median. The mean weight-adjusted AFP level for the trisomy 18 pregnancies was 1.64 multiples of the median. The mean AFP level for trisomy 18 pregnancies without a neural tube defect or abdominal wall defect was 1.37 multiples of the median. The median AFP level for all trisomy 18 pregnancies was 0.95 multiple of the median and 0.65 multiple of the median for those without a neural tube defect or abdominal wall defect. The cumulative distribution of the 13 retrospective cases and controls is shown in Fig. 1.

After conversion of the β-hCG levels to weight-adjusted multiples of the median on the basis of the median for each week of gestation, the distribution of β-hCG levels for cases and controls was analyzed. The mean β-hCG multiple of the median for cases was 2.78 and was 1.00 for controls. However, the median β-hCG for the trisomy 18 pregnancies was 0.27 multiple of the median. For those trisomy 18 pregnancies without a neural tube defect or abdominal wall defect, the mean β-hCG was 3.70 multiples of the median and the median was 0.32 multiple of the median. The cumulative distribution of β-hCG levels for the retrospective cases and controls is shown in Fig. 2.

After conversion of the unconjugated estriol levels to weight-adjusted multiples of the median on the basis of the median for each week's gestation, the distribution of unconjugated estriol levels for cases and controls were analyzed. The mean unconjugated estriol multiple of the median for cases was 0.65 and was 1.00 for controls. For those trisomy 18 pregnancies without a neural tube defect or abdominal wall defect, the mean unconjugated estriol was 0.63 multiple of the median and the median was 0.56 multiple of the median. The cumulative distribution of unconjugated estriol levels for the retrospective cases and controls is shown in Fig. 3.

Comment

Fetal trisomy 18 has been known to be associated with low levels of maternal serum AFP in the second trimester.[1, 8] Low levels of maternal serum were observed in trisomy 18 pregnancies by Bogart et al.[3, 4] and confirmed by Norgaard-Pedersen et al.[9] Canick et al.[6] recently analyzed data from 10 trisomy 18 pregnancies and confirmed low levels of maternal serum AFP, β-hCG, and unconjugated estriol in those pregnancies. They found median levels of AFP to be 0.57, β-hCG to be 0.27, and unconjugated estriol to be 0.49 multiple of the median, respectively. None of these 10 pregnancies had open fetal defects (neural tube defect or abdominal wall defect). Among our 10 pregnancies without open defects, the median levels are 0.65, 0.32, and 0.56 multiple of the median for AFP, β-hCG, and unconjugated estriol, respectively. These data appear to be similar to that of Canick et al.[6] However, if we include the four trisomy 18 pregnancies with open defects, the median levels become 0.95, 0.27, and 0.67 multiple of the median for AFP, β-hCG, and unconjugated estriol, respectively.

Among our 14 trisomy 18 pregnancies, there were three with markedly elevated β-hCG levels. This anomaly has not been observed or reported previously and may represent placental abnormalities associated with the trisomic state. We recently became aware of at least

two other cases of trisomy 18 pregnancies with β-hCG elevations reported by Blitzer et al.[10] In addition, two of the four trisomy 18 pregnancies with abdominal wall defect had maternal serum AFP levels within the normal range (0.58 and 0.65 multiple of the median). Thus the low maternal serum AFP level associated with trisomy 18 may mask the presence of an open fetal defect. We have also observed a normal amniotic fluid AFP in one trisomy 18 pregnancy associated with an abdominal wall defect (detected by routine amniocentesis and ultrasound for advanced maternal age).

In their article Canick et al.[6] suggest a combined cutoff of 0.75, 0.55, and 0.60 multiple of the median for AFP, β-hCG, and unconjugated estriol, respectively, leading to a 0.4% false-positive (amniocentesis) rate and a 60% detection rate.[6] Applying their recommendations to our cases would have lead to a 29% detection rate. Because four of our cases had elevated maternal serum AFP levels and would have been detected for that reason, a total of eight trisomy 18 pregnancies (57%) would have been detected by the Canick method.[6] If, instead, we were to use cutoffs of 0.70, 0.40, and 0.70 multiple of the median for AFP, β-hCG, and unconjugated estriol, respectively, we would have detected five of the 14 (36%) trisomy 18 pregnancies with a 0.3% false-positive rate. Added to the four pregnancies with elevated maternal serum AFP, the detection rate would be 9 of 14 (64%). If elevated β-hCG levels (≥2.5 multiples of the median) were also used, the detection rate would rise to 11 of 14 (79%). Thus the method and efficiency of trisomy 18 screening prospectively using the three markers need to be evaluated further.

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Ultrastructure of the microvasculature in the human endometrium throughout the normal menstrual cycle

Daniel K. Roberts, MD, PhD,[a] Tim H. Parmley, MD,[b] Nola J. Walker, EMSA,[a] and Douglas V. Horbelt, MD[a]

Wichita, Kansas, and Little Rock, Arkansas

In the early proliferative phase endometrial capillary basal lamina is loosely formed and discontinuous. It becomes more distinct in the late proliferative phase and develops whorled extensions that include pericytes in the early secretory to midsecretory phase. Throughout the cycle both endothelial cells and pericytes possess cytoplasmic processes that extend through discontinuities in the basal lamina and make contact with each other. These contacts may be simple or complex. They become more elaborate as the cycle progresses to the midsecretory phase. Endothelial cells show progressive but heterogeneous differentiation into the midsecretory phase, with individual cell hypertrophy. In the late-secretory phase the endometrial stroma and the basal lamina are characterized by widespread degeneration, and the cell-to-cell contacts sharply decrease. However, endothelial cells remain relatively intact. (Am J Obstet Gynecol 1992;166:1393-406.)

Key words: Human endometrial microvasculature, cell-to-cell interactions, pericytes, hypertrophy, endothelial cells

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6/1/34905

We have been interested in the morphologic ultrastructure of direct cell-to-cell contact in the human endometrium.[1,2] One place where such cell-to-cell contact might be expected would be in the endometrial microvasculature. Pericytes and endothelial cells are known to possess direct contacts in many other parts of the vascular tree.[3-5] Pericytes are known to have modulating effects on endothelial cell growth and differentiation,[5,6] and endothelial cell growth and differentiation

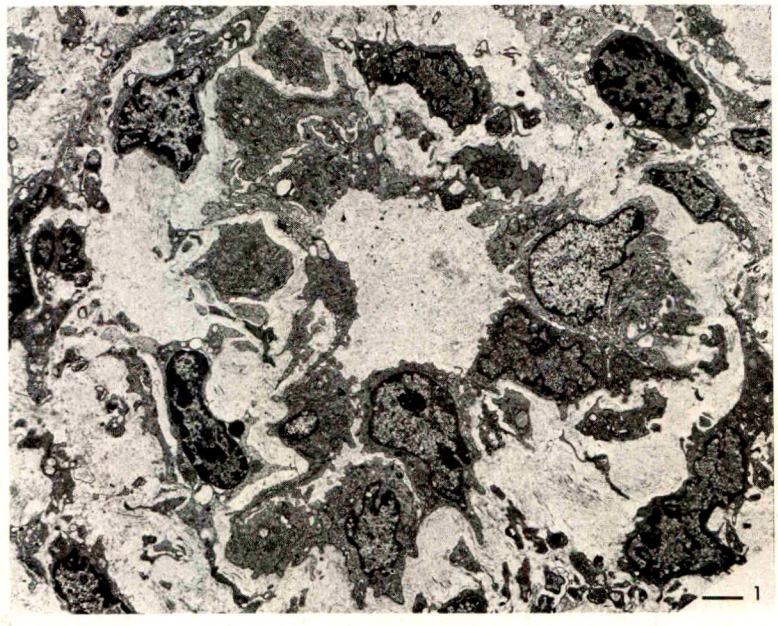


Fig. 1. Endothelial cells possess relatively little cytoplasm. They have smooth surfaces and the nuclear chromatin is clumped. (Original magnification ×3710. *Bar* 2.0 μm.)



Fig. 2. Developing Weibel-Palade bodies *(arrowheads)* are seen in association with Golgi. (Original magnification ×33,160. *Bar* 0.2 μm.)

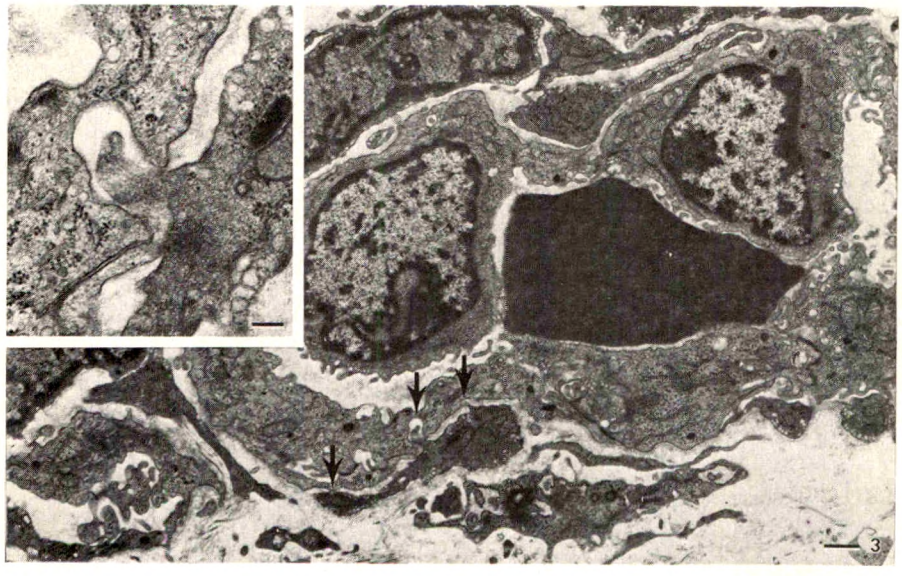


Fig. 3. Pericytes possess cytoplasmic extensions that make contacts with endothelial cells. The pericyte makes contact with adjacent endothelial cell at three sites *(arrows)*. (Original magnification ×6900. *Bar* 1.0 μm.) *Inset*, Central contact is the most elaborate. (Original magnification ×31,100. *Bar* 0.2 μm.)

presumably must take place cyclicly in the human endometrium.[7] Endothelial cell hypertrophy has also been described in experimental studies in endometrial capillaries.[8-11]

Therefore we studied pericyte-endothelial cell interactions and hypertrophy in the microvasculature of the human endometrium during the normal menstrual cycle.

Material and methods

Endometrial specimens were obtained by biopsy from normally menstruating women seen in the De-

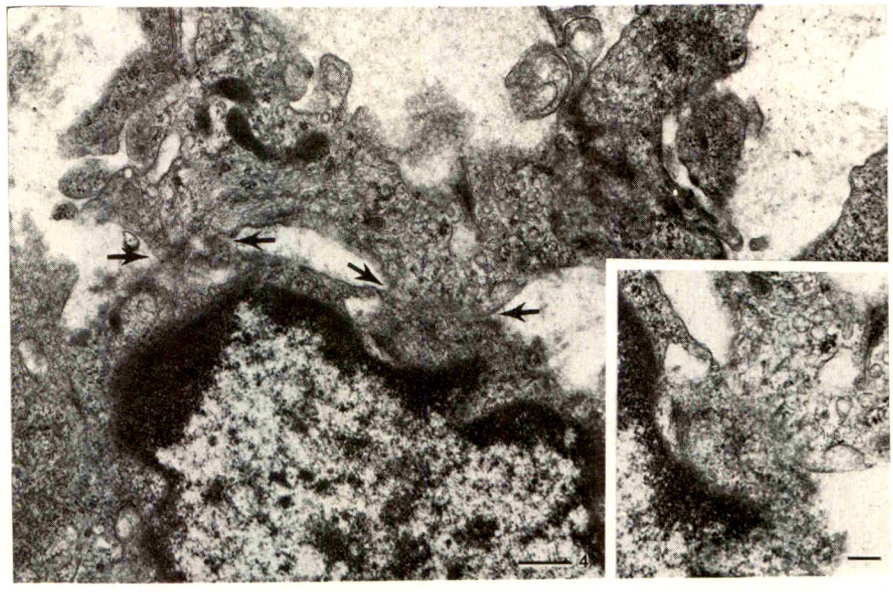


Fig. 4. An endothelial cell with intermediate filaments and pinocytotic vesicles makes diffuse contact with a pericyte at two points *(arrows)*. (Original magnification ×23,750. *Bar* 0.5 μm.) *Inset*, There is loss of definition of cell membranes approaching the site of contact, and filamentous structures appear to be continuous from one cell to the adjacent cell. (Original magnification ×30,375. *Bar* 0.2 μm.)

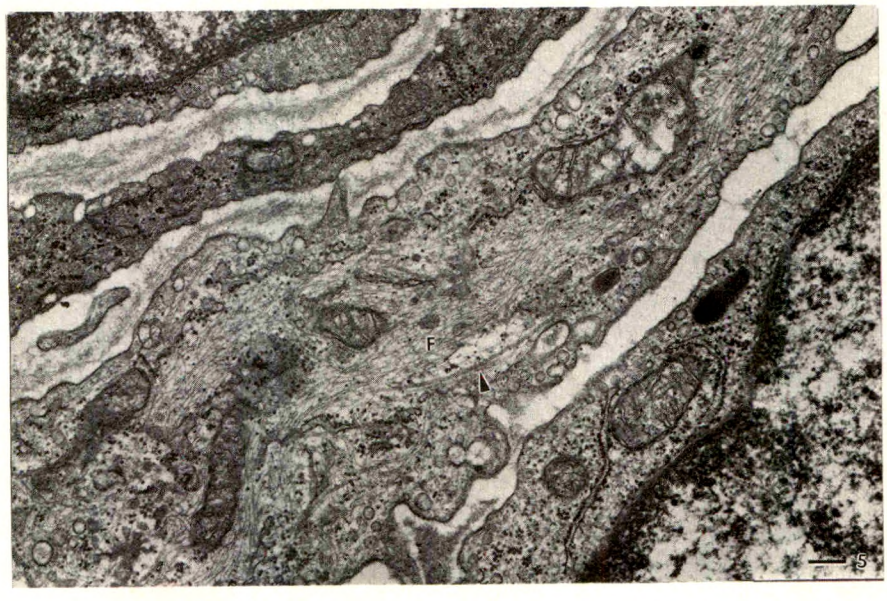


Fig. 5. Intermediate filaments *(F)* characterize endothelial cells. Microtubules are also present in the cytoskeleton *(arrowhead)*. (Original magnification ×28,500. *Bar* 0.2 μm.)

partment of Obstetrics and Gynecology at HCA-Wesley Medical Center during 1984 and 1985. Samples were obtained from five different women in each of the following phases of the cycle: early (days 5 to 9) and late (days 10 to 14) proliferative and early (days 15 to 19), middle (days 20 to 22), and late (days 23 to 28) secretory. None of the patients had received hormone preparations for 6 months before the biopsy procedure. Approximately half of each specimen was prepared for transmission electron microscopy. Hematoxylin and eosin–stained light microscopic samples were examined to confirm the presence of functionalis and to date the endometrial samples by the criteria of Noyes et al.[12]

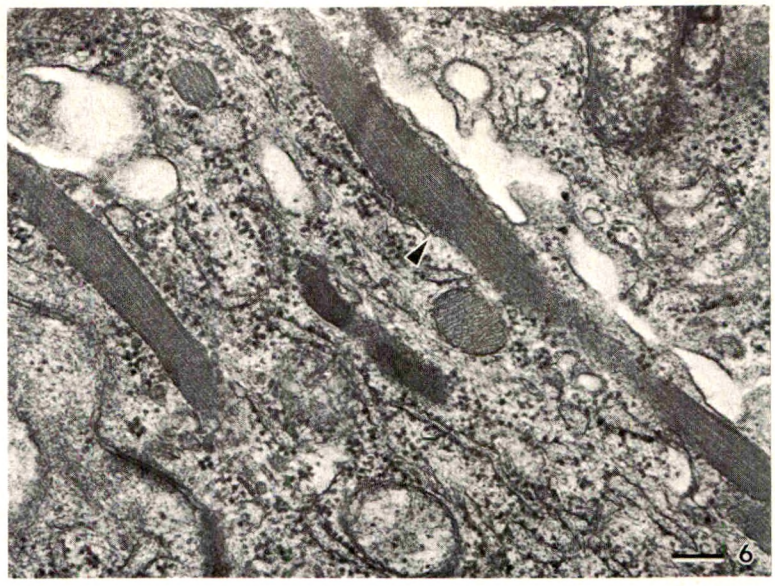


Fig. 6. More elaborate Weibel-Palade bodies *(arrowhead)* are seen in late proliferative phase endothelial cells. (Original magnification ×37,125. *Bar* 0.2 μm.)

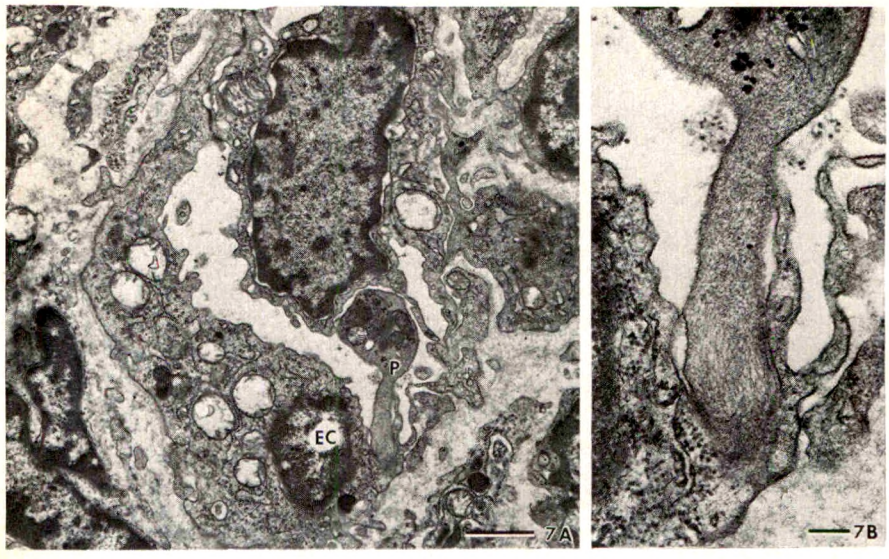


Fig. 7. A, In this late proliferative phase capillary a platelet *(P)* is attached to an endothelial cell *(EC)*. (Original magnification ×13,040. *Bar* 1.0 μm.) **B,** Attachment is characterized by loss of definition of cell membranes and dense filamentous connections. (Original magnification ×39,850. *Bar* 0.2 μm.)

Samples were prepared for transmission electron microscopy by routine methods.[13-16] Specimens were examined and photographed on a Philips 201 transmission electron microscope at magnifications ranging from ×3600 to ×121,000.

Initially, survey micrographs were taken of each sample, and capillaries were examined. Additional micrographs were taken, as required, to confirm the extent of an observation made in a given endometrium. A minimum of two tissue blocks (50 sections per block) were examined for each endometrial biopsy. Additional blocks were used as necessary to confirm the extent of any observed change.

Results

In the early proliferative phase nuclear chromatin in the endothelial cells is relatively condensed and the nuclei appear inactive (Fig. 1). Most of the endothelial

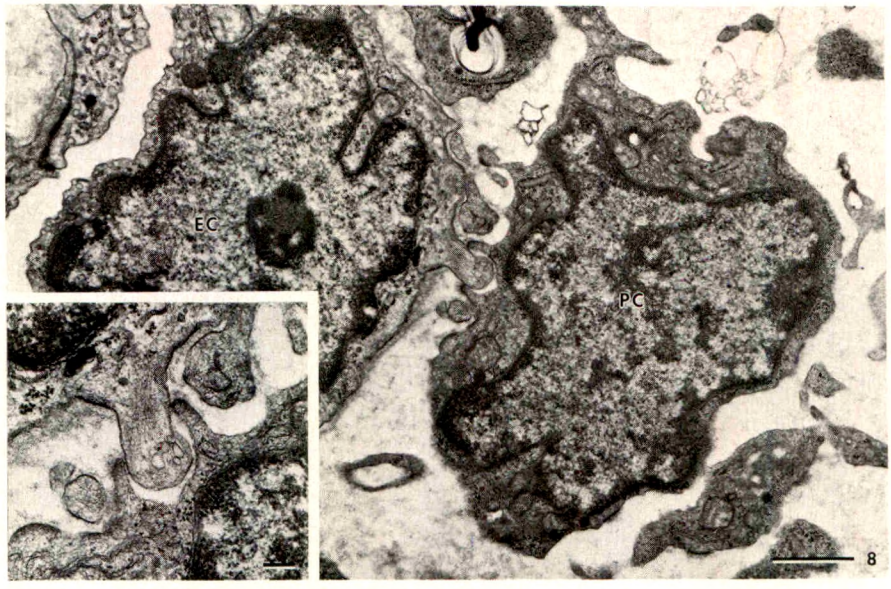


Fig. 8. This endothelial cell *(EC)* possesses a complex bulbous projection that contains both microfilaments and vesicles. The pericytic cell *(PC)* surface appears to be modified to accept this projection. (Original magnification ×16,200. *Bar* 1.0 μm.) *Inset,* The lips of the depression in pericytic cell surface make focal contact with shaft of endothelial cell projection.

cells possess only a small amount of cytoplasm, but they may be organelle rich. Weibel-Palade bodies, apparently arising from the Golgi apparatus, are present frequently (Fig. 2). There is a variable amount of Golgi and rough endoplasmic reticulum. Intermediate filaments and microfilaments are present. The luminal surface of the endothelial cells possesses only a few microvilli and is otherwise relatively smooth. Pinocytotic vesicles are common on both the basal and luminal surfaces. Endothelial cells are joined by tight junctions.

The stromal cells immediately surrounding the capillary, presumably pericytes, are of two types. One has large amounts of intracytoplasmic filaments and occasional dense bodies, thus resembling a smooth muscle cell. The other is fibroblastic in appearance. Both cell types have numerous pinocytotic vesicles. Vesicles are more frequent on the stromal surface of the smooth muscle–type cells.

The pericytes possess cytoplasmic extensions that project through the area of the basal lamina and make contact with the endothelial cells (Fig. 3, with inset). The capillary basal lamina is loosely formed and often discontinuous. Endothelial cells also possess cytoplasmic extensions that project through the area of the basal lamina to form connections with the pericytes.

These connections display varying degrees of intimacy. In some cases the cell membranes appear intact, and the site of contact consists of filaments connecting



Fig. 9. The lips of the cup-shaped depression are in contact with the base of pericytic cell cytoplasmic projection. The tip of the projection makes contact with the endothelial cell where pinocytotic vesicles apparently empty into the space containing the projection. (Original magnification ×47,250. *Bar* 0.2 μm.)

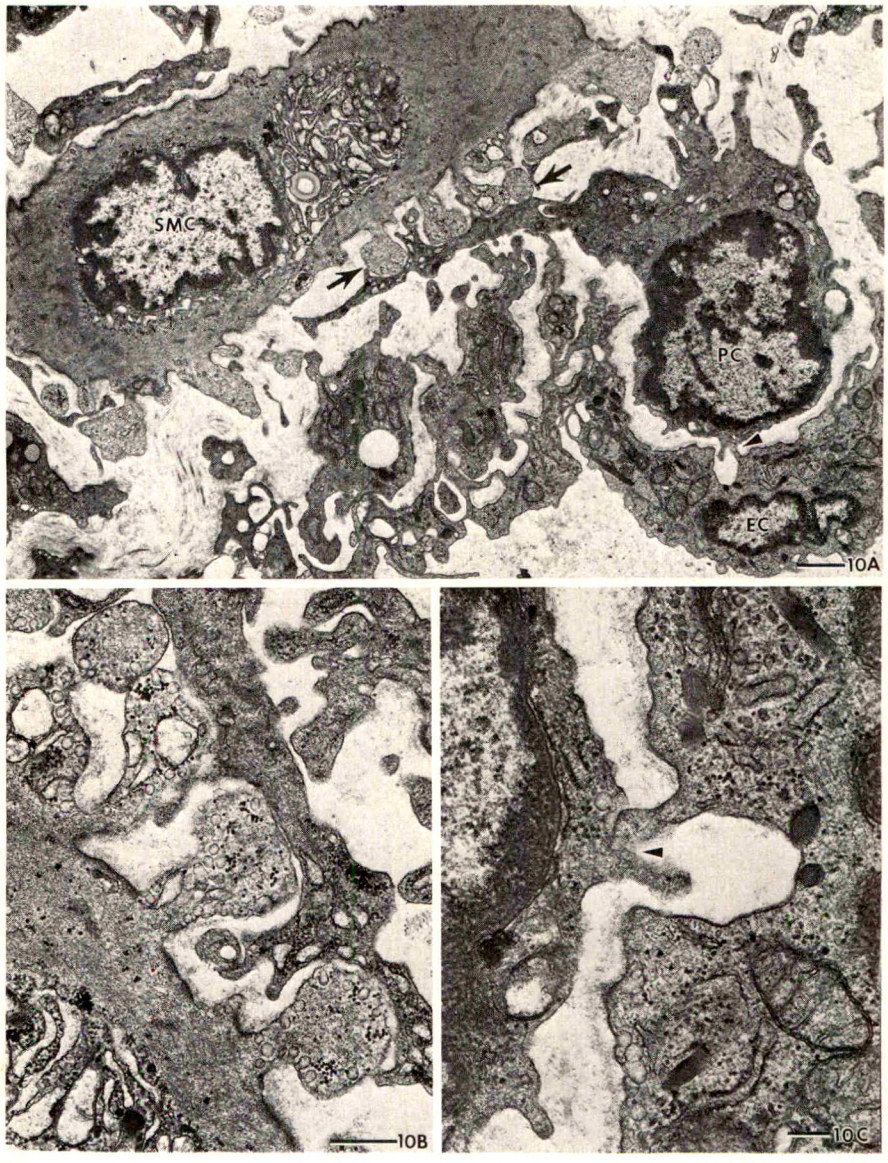


Fig. 10. A, Multiple cell-to-cell contacts *(arrows)* relate a smooth muscle–type cell *(SMC)* to a pericyte *(PC)*, which in turn makes contact *(arrowhead)* with an adjacent endothelial cell *(EC)*. (Original magnification ×9925. *Bar* 1.0 μm.) **B,** Numerous pinocytotic vesicles and glycogen granules occupy the tips of projections from smooth muscle–type cell. These tips appear to make contact with the pericyte. (Original magnification ×26,550. *Bar* 0.5 μm.) **C,** The pericyte also makes contact with endothelial cell *(arrowhead)*. (Original magnification ×40,500. *Bar* 0.2 μm.)

the two cell surfaces. Less commonly, the connections seem even more intimate, with cell membranes becoming indeterminate at the site of contact, and filaments in the cytoplasm of one cell are seemingly continuous with those in the cytoplasm of the adjacent cell (Fig. 4, with inset).

In the late proliferative phase many endothelial cell and pericyte nuclei become enlarged and are characterized by diffuse chromatin. Mitochondria are large and more numerous in both cell types. Rough endoplasmic reticulum is also more dilated and multiple Golgi possess more vesicles. In the endothelial cells both intermediate filaments and microfilaments are increased over the early proliferative phase (Fig. 5). Better-developed Weibel-Palade bodies are numerous (Fig. 6). Their relationship to the cell membrane is prominent.

Numerous platelets are seen in capillary lumina in

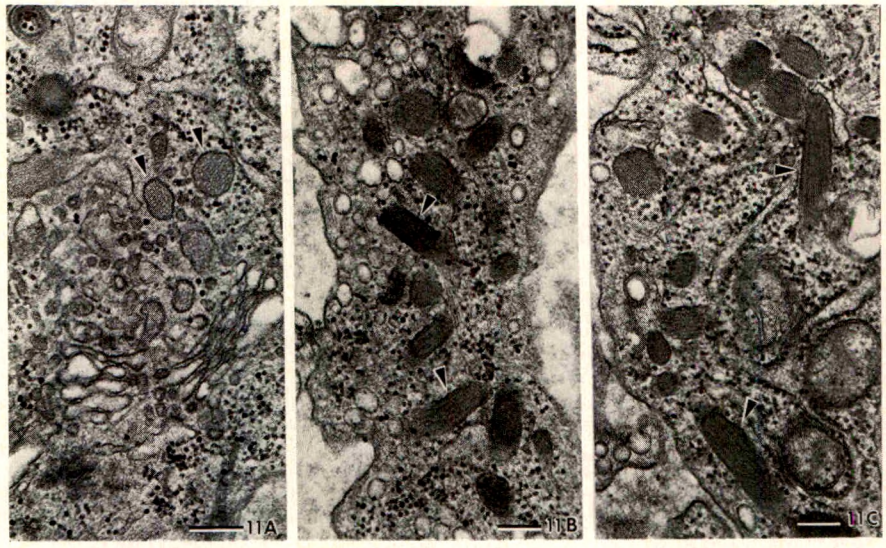


Fig. 11. A, Elaborate Golgi is closely associated with multiple Weibel-Palade bodies *(arrowheads).* (Original magnification ×22,570. *Bar* 0.5 μm.) **B** and **C,** Further stages of development in Weibel-Palade bodies are seen *(arrowheads).* (Original magnification ×42,070. *Bar* 0.2 μm.)

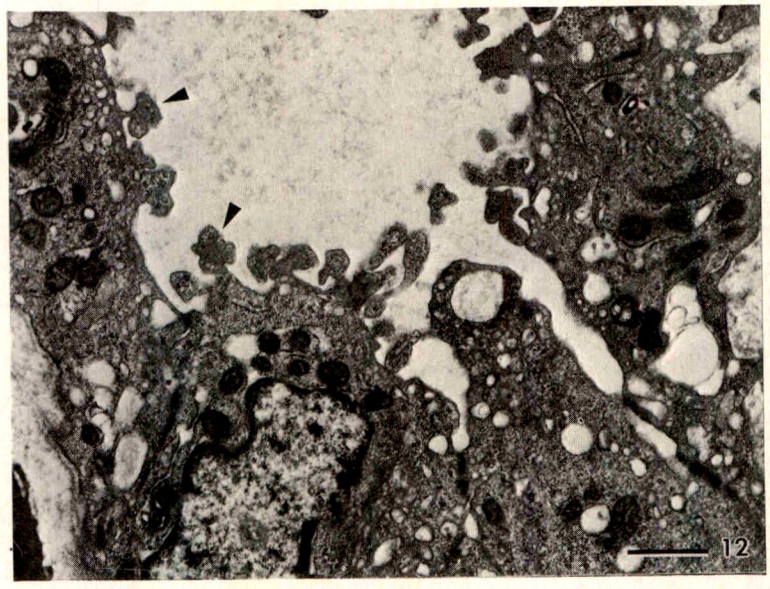


Fig. 12. Complex cytoplasmic blebbing *(arrowheads),* morphologically distinct from microvilli, characterize this endothelial cell surface. (Original magnification ×12,150. *Bar* 1.0 μm.)

the late proliferative phase. In one case an intraluminal platelet was seen to establish a fibrillar contact with an endothelial cell (Fig, 7, *A* and *B*). A search for fenestrations revealed one capillary with fenestrations in the basal lamina.

The pericytes are also more differentiated in the late proliferative phase. They possess enlarged nuclei with diffuse chromatin. Those pericytes that resemble smooth muscle cells are particularly prominent.

The capillary basal lamina is thickened and more diffuse than in the early proliferative phase. There is increased stromal collagen, which interacts with the endothelial basal lamina, pericytes, and surrounding stromal cells. Both the frequency and the complexity

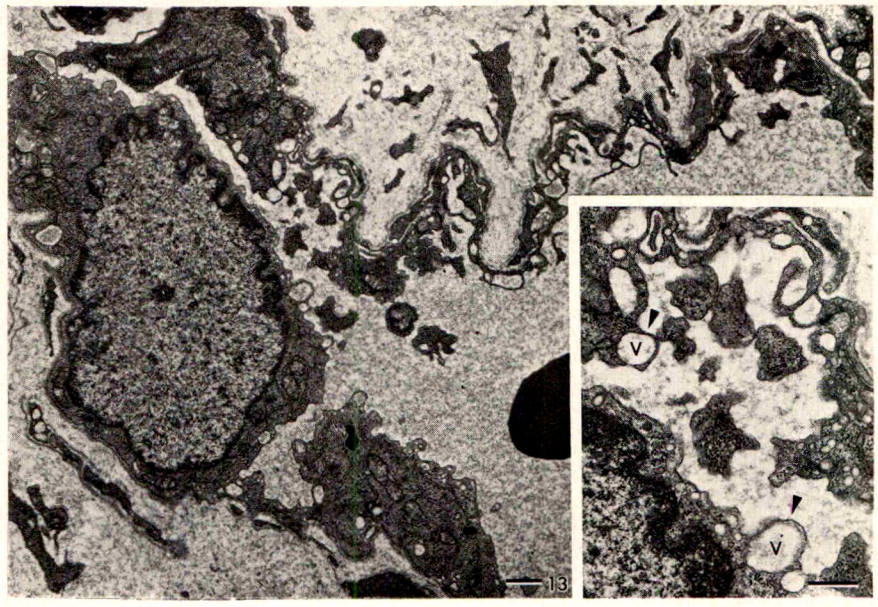


Fig. 13. Luminal surface of these capillary endothelial cells is characterized by microvilli, vacuoles, and surface blebs. (Original magnification ×7085. *Bar* 1.0 μm.) *Inset,* Pinocytotic vesicles are present but many of the large vacuoles *(V, arrowheads)* appear to be formed by microvilli that have folded back and fused with the cell surface. (Original magnification ×19,125. *Bar* 0.5 μm.)

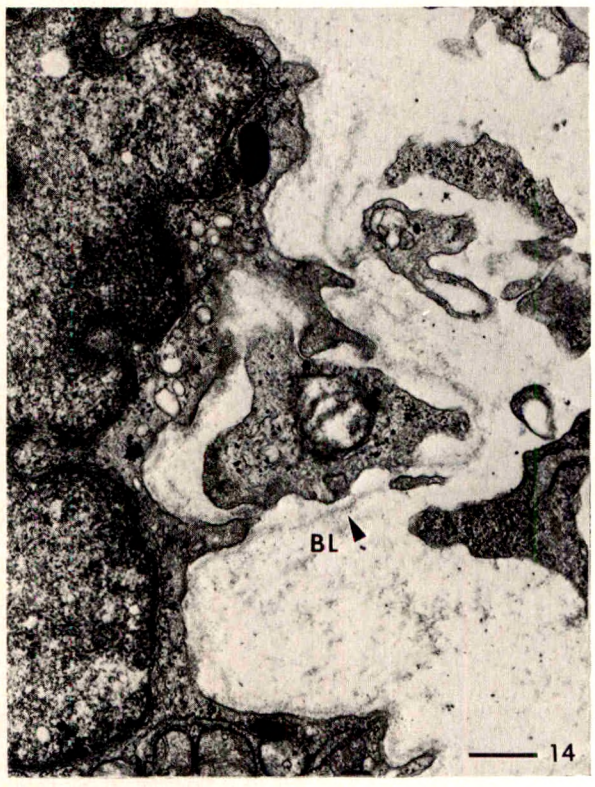


Fig. 14. Basal lamina *(BL, arrowhead)* is developing laminations and beginning to envelop a pericyte. (Original magnification ×20,200. *Bar* 0.5 μm.)

of direct connections between endothelial and pericytic cells appear to be increased. Endothelial cell cytoplasmic projections extend into indentations and make contact with stromal cells in a variety of ways. These were described in the early proliferative phase but have become more elaborately developed. For example, endothelial cell cytoplasmic projections may possess bulbous tips that appear to fit into indentations in the pericytic cell surface (Fig. 8, with inset).

Also, cytoplasmic projections from pericytic cells extend into the endothelial cell compartment through discontinuities in the basal lamina. The tip of the pericytic cell projection extends into the cup-shaped depression of the endothelial cell. There is often contact between the lips of the endothelial cell cup and the base of the pericytic cell projection (Fig. 9). Pinocytosis is often observed in the base of this cup-shaped depression. Heterogeneous connections are seen when a smooth muscle–type cell possessing a series of bulbous cytoplasmic projections fit into cytoplasmic indentations of an adjacent pericyte and in turn makes contact with an endothelial cell (Fig. 10, *A* through *C*).

In the early secretory phase of the cycle selected endothelial cells begin to experience cytoplasmic growth. This growth may occur in one endothelial cell and be absent in an adjoining cell. Where growth occurs, there are substantial increases in Golgi, rough endoplasmic reticulum, and vacuoles. The Golgi becomes more elab-



Fig. 15. Two of the endothelial cells in this capillary are dramatically enlarged with increased ribosomes and other organelles. Other endothelial cells in the capillary are apparently not participating in this process. (Original magnification ×7085. *Bar* 1.0 μm.)

orate and the rough endoplasmic reticulum begins to dilate. Weibel-Palade bodies in varying stages of development persist (Fig. 11, *A* through *C*). Both endothelial cells that enlarge and those that do not show increased surface blebbing (Fig. 12), microvilli, and vacuoles apparently formed by microvilli falling back onto the cell surface[17] (Fig. 13, with inset). Both cell types also seem to have less active nuclei than in the late proliferative phase, with more clumped chromatin and smaller size.

At the same time both the fibroblastic and smooth muscle–type pericytic cells participate in the morphologic process termed decidualization. An increased number of ribosomes, dilated rough endoplasmic reticulum, vacuoles, and more elaborate Golgi complexes develop, contributing to increased cell size. Light glycogen also appears. Basal bodies continue to appear in stromal cells and, in a few cases, cilia are formed. The pericytes resembling smooth muscle cells that contain large amounts of microfilaments and dense bodies are less apparent. They now possess many more cell organelles, and the filaments appear diluted.

The basal lamina becomes significantly more intact and also more complex. It begins to develop laminations and starts to envelop small pericytes (Fig. 14). Although the number of direct communications between endothelial and pericytic cells seems decreased in this phase, remaining connections are more elaborate.

In the midsecretory phase those endothelial cells that are enlarging have now done so, even more dramatically, and may almost fill a capillary space. This process remains heterogeneous (Fig. 15). Free ribosomes, mitochondria, dilated rough endoplasmic reticulum, and elaborate Golgi complexes are present. Weibel-Palade bodies are frequent and well developed in this phase, as are intermediate filaments. The endothelial surface is somewhat less complex.

The pericytic cells are even more decidualized than in the early secretory phase. Basal bodies are prominent and rare cilia are present.

The basal lamina becomes better defined than in any other time in the cycle. It is profusely elaborate with many additional lamina enveloping as many as two or three layers of pericytic cells (Fig. 16, *A* through *C*). Cytoplasmic interdigitations between endothelial cells and stromal cells are more evident than in the early secretory phase. In addition, larger surface areas of each cell are involved in these cell contacts (Fig. 17, with inset). More endometrial stromal cells consistent with the endometrial granulocyte are seen immediately adjacent to the capillary in a pericytic position (Fig. 18). Cell-to-cell interactions between this cell type and the endothelial cells are also seen (Fig. 18, inset).

In the late secretory phase there is widespread degeneration in the stromal compartment of the endometrium, consisting of extensive cellular fragmentation. The capillary basal lamina is much more fragmented. There is a marked decrease in the interactions between endothelial and pericytic cells.

However, the endothelial cells, in spite of apparent separations, are characterized by their contrast with the fragmenting cells in the stroma (Fig. 19). In many cases

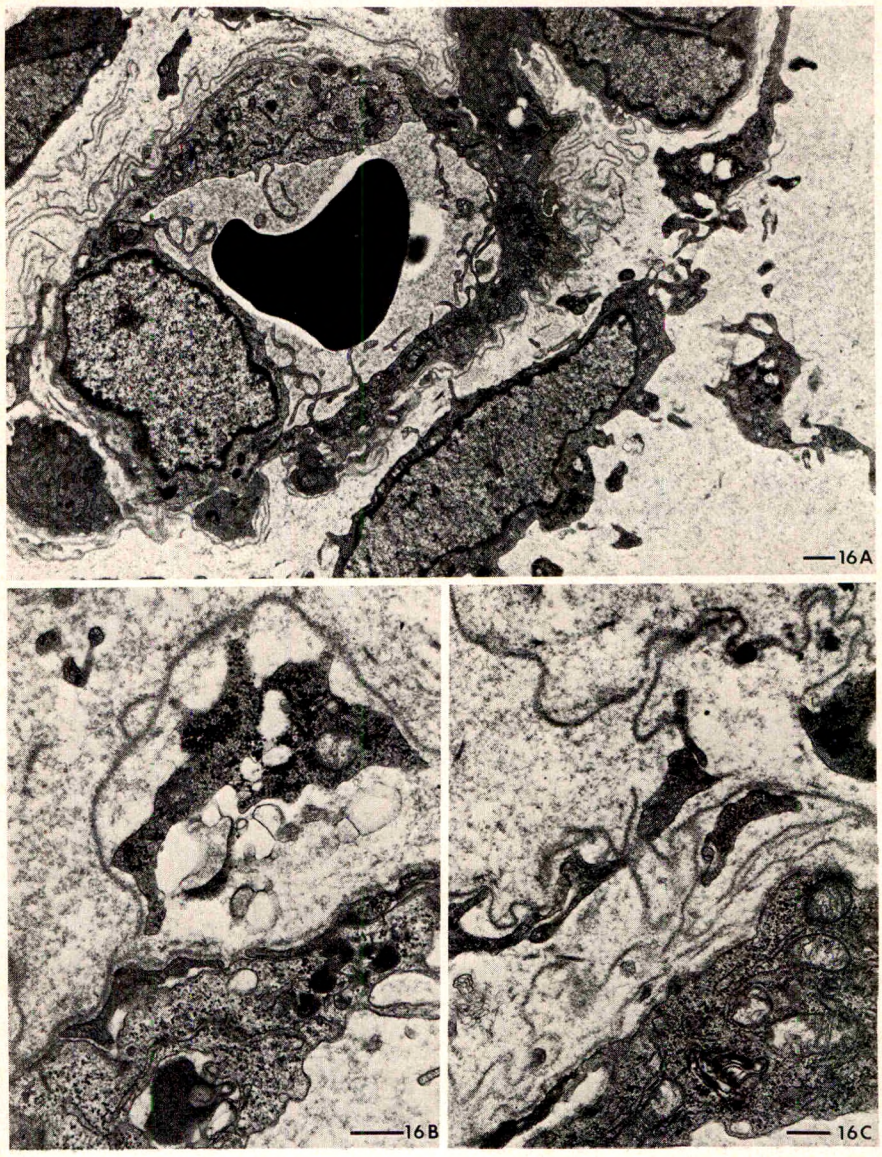


Fig. 16. Basal lamina possess many laminations, some of which envelop adjacent cells, whereas others are arranged as whorls in the extracellular space. (**A,** Original magnification, ×6900; *bar* 1.0 μm. **B,** Original magnification, ×22,450; *bar* 0.5 μm. **C,** Original magnification, ×17,225; *bar* 0.5 μm.)

the endothelial cells appear quite healthy and, in some instances, even more hypertrophied than in the midsecretory phase. There is an apparent increase in the number of free ribosomes, rough endoplasmic reticulum in long strands, and Golgi complexes. Frequently multiple small Weibel-Palade bodies are associated with this elaborate Golgi. Weibel-Palade bodies are often found at the tips of cytoplasmic projections, both into the lumen of the capillary (Fig. 20, *A*) and occasionally near the abluminal plasma membrane as well (Fig. 20, *B*). The endothelial cell surfaces show scattered microvilli, and fibrin is readily identified in the capillary lumen (Fig. 21, *A* and *B*). There is also an increased number of multivesicular bodies in the late secretory capillary endothelial cells. These bodies display heterogeneity of form and increased numbers.

Comment

This report, as well as two previously published, allows for a composite reconstruction of the ultrastructure of cell-to-cell contact in the human endometrium.[1,2] From the endothelial cell through stromal cells to the epithelium, the tissue is continuous. This includes the endometrial granulocyte that interacts with other endometrial cell types. The sites of contact are relatively small in the early proliferative phase. They increase in

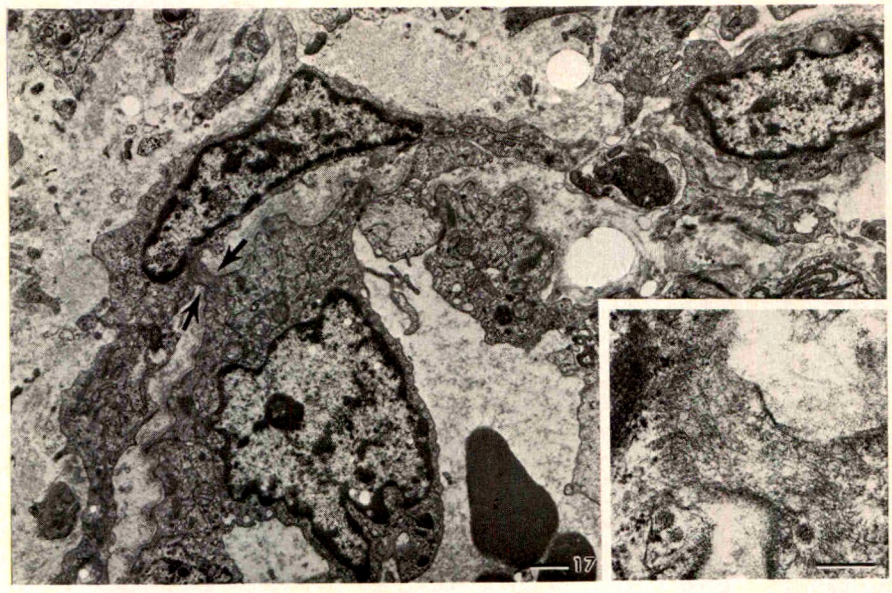


Fig. 17. An endothelial cell projection makes contact with a pericyte *(arrows)*. (Original magnification ×7950. Bar 1.0 μm.) *Inset*, Cell membrane definition is lost approaching this site, and filamentous structures appear to be continuous from one cell to another. (Original magnification ×45,350. *Bar* 0.25 μm.)

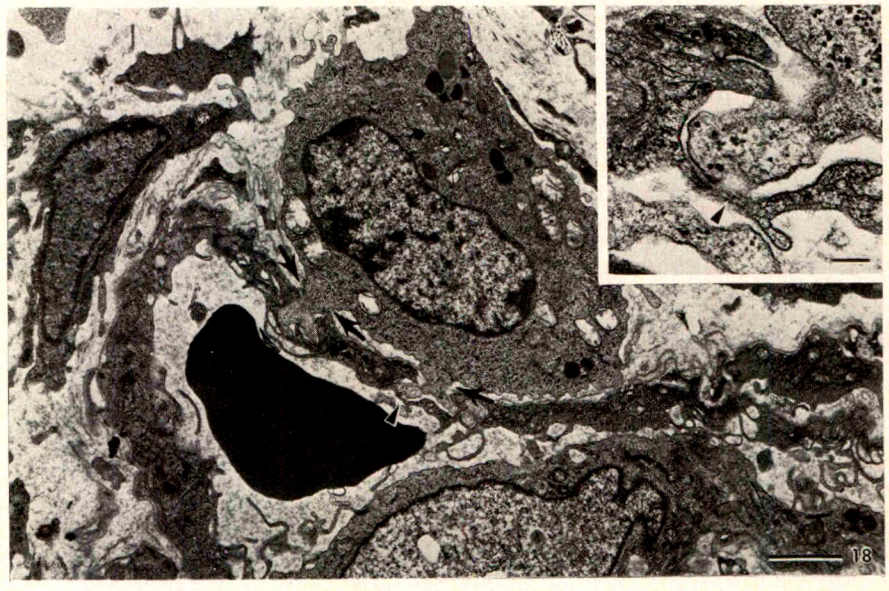


Fig. 18. This endometrial granulocyte, possessing both granules and a kidney bean–shaped nucleus, also possesses two cytoplasmic extensions into the endothelial cell compartment *(arrows)*. Both projections divide and become more complex as they enter the endothelial cell compartment. (Original magnification ×7250. *Bar* 2.0 μm.) *Inset*, One of the resulting papillae deeply penetrates the endothelial cell, forming close cell-to-cell contact *(arrowheads)*. (Original magnification ×37,125. *Bar* 0.2 μm.)

number and complexity and are extensive by the end of the proliferative phase. During the early and midsecretory phases connections between all microvascular cell types become more elaborate in structure and larger in size, although possibly they are less frequent. In the late secretory phase these connections become even less frequent.

A wide variety of evidence supports the idea that cell-to-cell contact is important in the control of differentiation. In mammalian embryos cells with similar de-

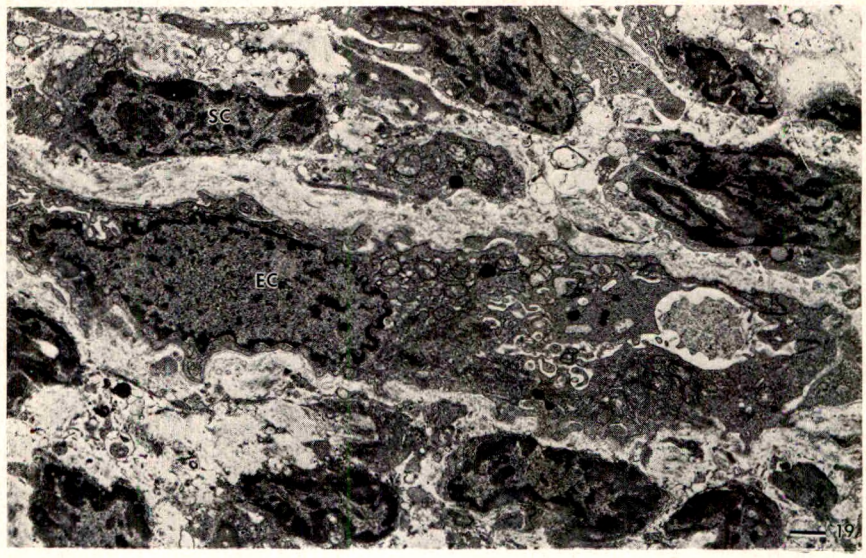


Fig. 19. Endothelial cells *(EC)* are substantially more intact than surrounding stromal cells *(SC)*. (Original magnification ×7085. *Bar* 1.0 μm.)

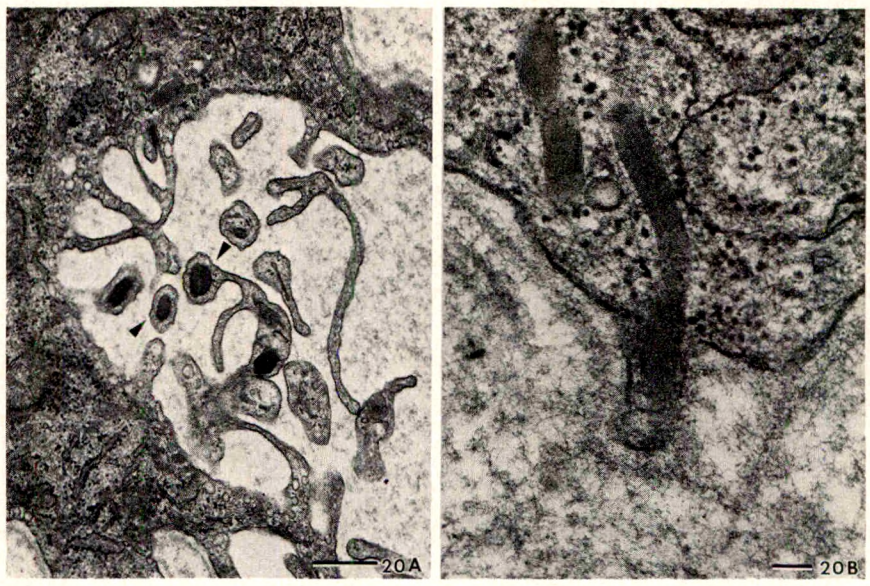


Fig. 20. A, Endothelial microvilli possess Weibel-Palade bodies *(arrowheads)* in the tips extending into the lumen of capillary. (Original magnification ×25,920. *Bar* 0.5 μm.) **B,** A Weibel-Palade body is also seen projecting through the abluminal plasma membrane. (Original magnification ×75,940. *Bar* 0.1 μm.)

velopmental fates remain connected by gap junctions, whereas those with divergent developmental fates lose these connections.[18] The induction of mesoderm from embryonic ectoderm by endoderm in the mouse is known to require close cellular proximity.[19] Pericytes modulate endothelial cell behavior and are most effective in this regard when in contact with endothelial cells.[6] Recently, epidermal growth factor has been demonstrated at the site of pericyte projections into endothelial cells.[20]

Endometrial epithelial cells are known to require endometrial stroma to display some types of normal behavior, including differentiation.[21-23] Consequently, it seems plausible that the coordinated growth and de-

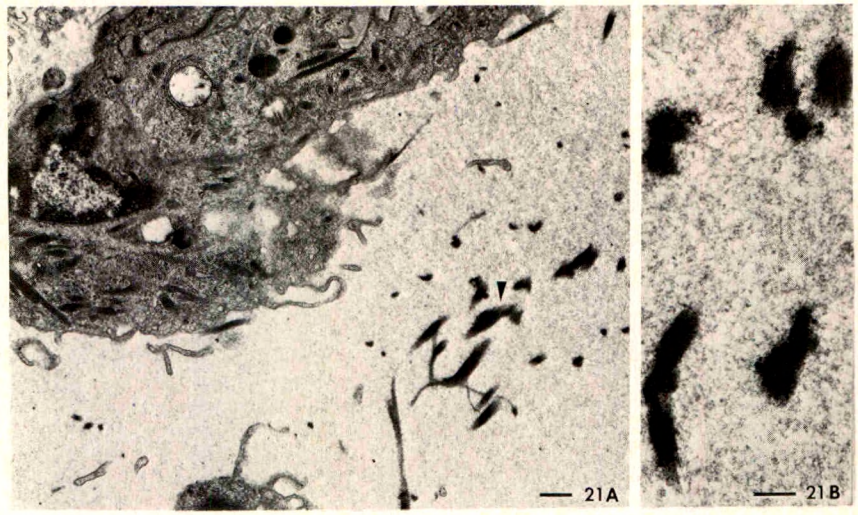


Fig. 21. A, Large amounts of fibrin *(arrowhead)* are present in this capillary lumen. (Original magnification ×6910. *Bar* 1.0 μm.) **B,** Fibrin is seen here at higher magnification. (Original magnification ×40,500. *Bar* 0.2 μm.)

velopment of the endometrium is, at least in part, supported by the extensive physical contacts that connect its component cell types.

Endothelial cell hypertrophy has been described in the endometrial capillaries of several primates, including human, at implantation sites.[8-11] Potential confusion of this phenomenon with the trophoblast invasion of vasculature at implantation sites described by Ramsey,[24] led Denker et al.[8] to perform an experimental study that excluded the trophoblast. When a silk suture and progesterone were combined in the endometrium of the rhesus monkey, endothelial cell hypertrophy was noted. Wilborn and Flowers[25] reported a similar change in the capillaries of the endometria of women receiving estrogen-progestin–containing oral contraceptives. We have seen endothelial cell hypertrophy in the endometrial capillaries of women during the luteal phase of the normal cycle. Therefore it seems logical to attribute this phenomenon to progesterone in combination with estrogen. One can only speculate about the function or functions of these cells, but it is now commonly accepted that endothelial cells are involved in many physiologic functions beyond simply lining the capillaries.[26]

A striking observation is that capillary endothelial cells seem quite healthy in late secretory endometria, in spite of considerable necrosis in the tissues around them. This would seem to support a continuous role for the capillaries in the phenomenon of menstruation. Wagner and Marder[27] used biochemical observations to substantiate the concept that Weibel-Palade bodies[28, 29] serve as storage or processing organelles for von Willebrand factor. In addition, it is believed that fibrin may be involved in von Willebrand factor release from Weibel-Palade bodies.[30] The increased activity in Weibel-Palade bodies and increasing amounts of intraluminal fibrin in late secretory endometrium may be evidence for their combined roles in menses. Determination of a relationship between von Willebrand factor and menstruation would be most interesting.

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PROGRAM

THURSDAY, SEPTEMBER 24, 1992

7:00 A.M.: Registration
7:45 A.M.: Welcome/S. Robert Kovac, M.D.
7:55 A.M.: Stephen H. Cruikshank, M.D. (Scientific Moderator)
8:00 A.M.: Lane J. Mercer, M.D. *"Antibiotic Prophylaxis for Vaginal Surgery—How Much, When"*
8:30 A.M.: S. Joel Joel-Cohen, M.D. *"General Surgical Principles as Applied to Vaginal Surgery"*
9:00 A.M.: George W. Morley, M.D. *"Vaginal Hysterectomy—How I Do It"*
9:30 A.M.: Break
9:50 A.M.: Thomas G. Stovall, M.D. *"Vaginal Hysterectomy—Tips and New Ideas to Include Outpatient Vaginal Hysterectomy"*
10:20 A.M.: John H. Isaacs, M.D. *"The Difficult Vaginal Hysterectomy"*
10:50 A.M.: David S. Chapin *"Combined Coring and Bisecting for Large Uteri"*
11:20 A.M.: Question & Answer Period
Noon: Lunch
1:00 P.M.: Bobby L. Shull, M.D. *"Anterior Vaginal Segment—Function and Anatomy"*
1:30 P.M.: David W. Warrell, M.D. *"Combined Assessment of Pelvic Visceral Function"*
2:00 P.M.: Marvin H. Terry Grody, M.D. *"Normal Vaginal Axis—Importance of Restoration in Pelvic Support"*
2:30 P.M.: Richard C. Bump, M.D. *"Effect of Vaginal Hysterectomy on Lower Urinary Tract Function"*
3:00 P.M.: Break
3:20 P.M.: Stephen H. Cruikshank, M.D. *"Ureteral Anatomy at Vaginal Hysterectomy"*
3:50 P.M.: Roundtable
Moderators: S. Robert Kovac, M.D.—Stephen H. Cruikshank, M.D.
"Vaginal Hysterectomy: To Close Or Not To Close Peritoneum—Transverse or Vertical Vaginal Cuff Closure—How to Support Vaginal Cuff"
George W. Morley, M.D. John H. Isaacs, M.D.
Bobby L. Shull, M.D. Thomas G. Stovall, M.D.
4:30 P.M.: Question & Answer Period
5:00 P.M.: Adjourn
Wine and Cheese Reception/Exhibit Hall

FRIDAY, SEPTEMBER 25, 1992

7:30 A.M.: Update/Stephen H. Cruikshank, M.D.
8:00 A.M.: John H. Isaacs, M.D. *"Complications of Vaginal Hysterectomy and Repair"*
8:30 A.M.: Bobby L. Shull, M.D. *"Technical Aspects and Results of Paravaginal Defects—Including Vaginal Paravaginal Repair"*
9:00 A.M.: David S. Chapin, M.D. *"Teaching Sacrospinous Suspension—Exposing the Ligament So All Can See"*
9:30 A.M.: Break
10:00 A.M.: George W. Morley, M.D. *"Total Colpocleisis as Treatment of Vaginal Prolapse in the Elderly"*
10:30 A.M.: Marvin H. Terry Grody, M.D. *"The Dysfunctional Postoperative Vagina"*
11:00 A.M.: W. Glenn Hurt, M.D. *"Vaginal Vault Prolapse—Treatment Options"*
11:30 A.M.: Question & Answer Period
Noon: Lunch
1:00 P.M.: S. Joel Joel-Cohen, M.D. *"The Story of Vaginal Surgery"*
1:30 P.M.: *"Vaginal Surgeon's Award"*
Presented by: S. Robert Kovac, M.D.
Presented to: S. Joel Joel-Cohen, M.D.
1:35 P.M.: S. Robert Kovac, M.D. *"Surgical Management of Uterine Prolapse in Young Women"*
2:05 P.M.: Lane J. Mercer, M.D. *"Complications of Episiotomy"*
2:35 P.M.: Harry Reich, M.D. *"Laparoscopic Hysterectomy—Definition and Technique"*
3:05 P.M.: Break
3:20 P.M.: Thomas G. Stovall, M.D. *"Laparoscopic Assisted Vaginal Hysterectomy"*
3:50 P.M.: Harry Reich, M.D. *"Laparoscopic Hysterectomy when Vaginal Hysterectomy is Impossible"*
4:20 P.M.: Roundtable
Moderators: S. Robert Kovac, M.D.—Stephen H. Cruikshank, M.D.
"Laparoscopy Assisted Vaginal Hysterectomy—Laparoscopic Assisted Vaginal Hysterectomy—Laparoscopic Hysterectomy: Valuable Methods—Valuable Marketing Tools"
Harry Reich, M.D. Marvin H. Terry Grody, M.D.
Thomas G. Stovall, M.D.
5:00 P.M.: Question & Answer Period
5:30 P.M.: Adjourn
7:00 P.M.: Social Dinner/Dance

SATURDAY, SEPTEMBER 26, 1992

7:30 A.M.: Update/S. Robert Kovac, M.D.
7:45 A.M.: W. Glenn Hurt, M.D. *"Urogynecology—Training and Practice"*
8:15 A.M.: David W. Warrell, M.D. *"Aetiology of Stress Incontinence of Urine and Surgical Management"*
8:45 A.M.: Donald R. Ostergard, M.D. *"Selection of the Appropriate Surgical Procedure"*
9:15 A.M.: Break
9:45 A.M.: Richard C. Bump, M.D. *"Role of Periurethral Bulk Injections in the Treatment of Incontinence"*
10:15 A.M.: Donald R. Ostergard, M.D. *"Why Surgery Cures or Fails"*
10:45 A.M.: David W. Warrell, M.D. *"The Management of Lower Urinary Tract Fistulae"*
11:15 A.M.: Roundtable
Moderators: S. Robert Kovac, M.D.—Stephen H. Cruikshank, M.D.
"Urogynecology: Operative Choices for Recurrent Stress Urinary Incontinence"
David W. Warrell, M.D. Donald R. Ostergard, M.D.
Richard C. Bump, M.D. W. Glenn Hurt, M.D.
Noon: Question & Answer Period
12:30 P.M.: Adjourn

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Chorionic villus sampling for fetal Rh typing: Clinical implications

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OBJECTIVE: The prenatal Rh typing of red blood cells obtained by chorionic villus sampling was performed with an immune rosette technique.

STUDY DESIGN: Ten Rh-negative pregnant women undergoing chorionic villus sampling at 9 to 11 weeks' gestation were studied at a large referral hospital.

RESULTS: Accurate Rh phenotyping may be done on red blood cells obtained from chorionic villi weighing 2 to 8 mg. The preparations were shown to be >90% fetal in origin as demonstrated by radial immunodiffusion quantitation of fetal hemoglobin–containing cells. Of the 10 fetuses typed in the first trimester nine of the pregnancies were carried to term. In all cases typing of red blood cells confirmed the antenatal fetal red cell typing.

CONCLUSIONS: This study shows that antenatal Rh phenotyping may be performed as early as 9 to 11 weeks' gestation. This technique may be used in the management of pregnancies complicated by Rh incompatibility. (Am J Obstet Gynecol 1992;166:1407-11.)

Key words: Hemolytic disease of the newborn, chorionic villus sampling, prenatal diagnosis

Prenatal diagnosis of genetic disorders in the second trimester of pregnancy by amniocentesis is a well-established technique. The procedure was actually first used in 1950 for the obstetric management of Rh disease. Recently, chorionic villus sampling has evolved as a useful clinical procedure for the diagnosis of genetic disorders.[1] Chorionic villus sampling can be performed during the first trimester of pregnancy (9 to 12 weeks' gestation), with earlier test results allowing termination of pregnancy during this safer time period.

In spite of the standard use of Rh immune globulin prophylaxis in Rh-negative mothers with Rh-positive fetuses, Rh immunization still occurs. The management of fetuses at risk for severe hemolytic disease of the newborn requires intensive monitoring with amniocentesis and ultrasonography. Therapeutic measures include intrauterine transfusions, intravenous γ-globulin, plasmapheresis, and early delivery.[2] We show that chorionic villus sampling very early in pregnancy can reliably identify the Rh status of fetuses. This may lead to the improvement of antepartum management of immune hemolytic disease of the newborn.

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Material and methods

Isolation of fetal red blood cells. Chorionic villus samples weighing 2 to 8 mg were obtained under ultrasonographic guidance in accordance with Johns Hopkins Maternal-Fetal Tissue guidelines and with the informed consent of the patient. Samples were collected from 10 Rh-negative mothers undergoing chorionic villus sampling at 9 to 11 weeks' gestation for genetic analysis. Blood samples were collected from the women before the procedure, and all women received Rh immunoglobulin prophylaxis after the procedure.

The chorionic villus samples were collected in Hanks' balanced salt solution (Gibco, Grand Island, N.Y.). Removal of surface-contaminating maternal red blood cells was done by gently washing the tissue in isotonic saline solution. The fetal red blood cells were then harvested by crushing the villi through a fine mesh screen suspended over a microtiter well (Immulon I Removawell, Dynatech Laboratories, Chantilly, Va.). The microwell was then placed in a 12 × 75 mm tube and the cells washed three times with saline solution. After each wash the saline solution was removed with a Pasteur pipet. The use of the microtiter wells facilitated handling of the 100 μl samples and reduced the risk of sample loss. We found that the villus samples stored at 4° C for 5 days in Hanks' balanced salt solution before processing still gave reliable results.

To validate that the harvested cells were of fetal origin, fetal hemoglobin-bearing blood cells were measured by a microscopic single-cell radial immunodif-

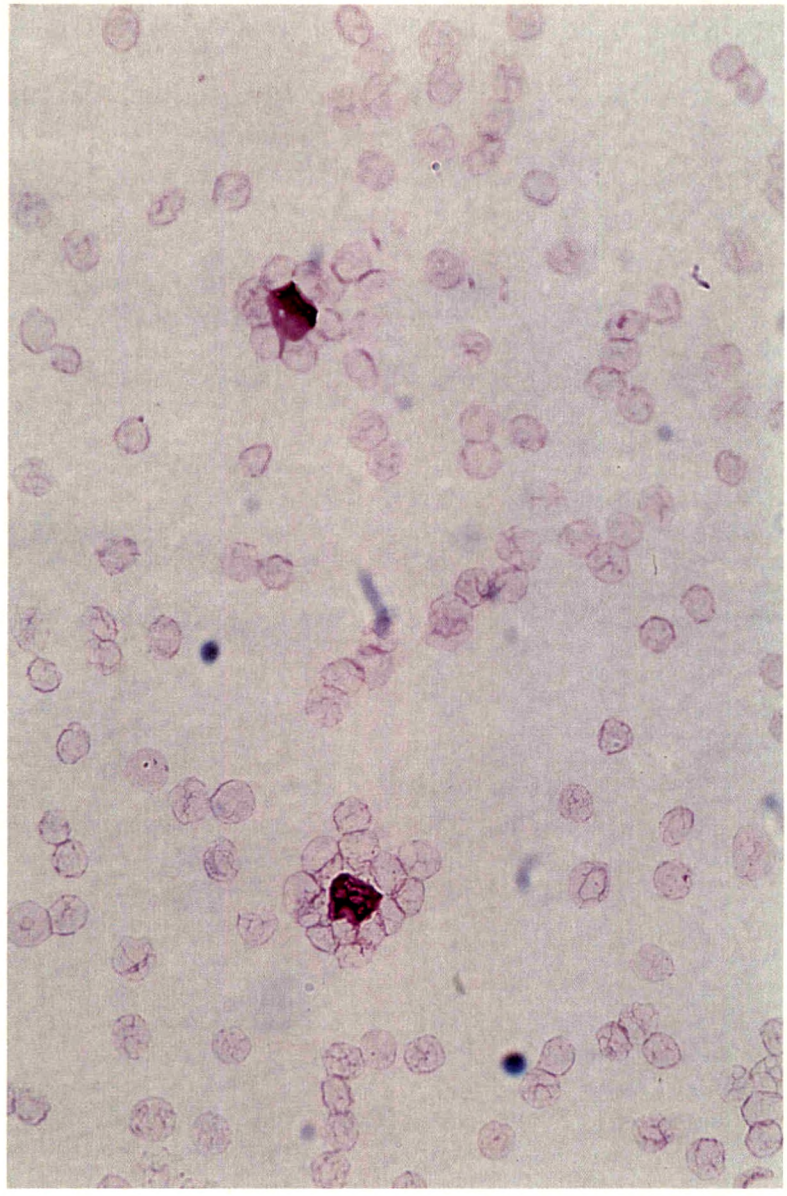


Fig. 1. Rosette that has been stained for fetal hemoglobin. In center of field is dark-staining fetal cell surrounded by pale-staining indicator cells.

fusion method using a rabbit antihuman fetal hemoglobin antibody.[3] Some preparations were stained for fetal hemoglobin with the F Cell Stain kit (Simmler, St. Louis).

Immune rosette test. To perform the immune rosette test, 50 μl of anti-D and 50 μl of papain (Organon Teknika Corp., Parsippany, N.J.) were added to the isolated fetal cells. Controls consisting of 1% suspensions of D-positive cells mixed with D-negative cells (0.5%, 0.25%, and 0%) were tested in parallel. The tests and controls were incubated at 37° C for 30 minutes and then washed six times with saline solution. After the addition of 50 μl of a 1% saline suspension of papain-pretreated D-homozygous (R_2R_2) cells and centrifugation, the cells were gently resuspended and placed on slides for microscopic observation.

The results are expressed as the number of rosettes per high-power field. At least one rosette per high-power field was the criterion of positivity. A rosette was defined as five or more indicator cells adherent circumferentially to a fetal red blood cell. Approximately 10 microscopic fields were examined by two independent readers.

Results

The number of red blood cells harvested per villus sample ranged from 10^6 to 10^8. Chorionic villus samples weighing as little as 2 mg yielded sufficient fetal red

Table I. Results of chorionic villus Rh typing and cord blood Rh phenotyping in 10 cases

Case No.	*Weight of villus (mg)*	*Chorionic villus Rh type*	*Rosettes (No./high-power field)*	*Cord Rh phenotype*
1	5	D+	2	D+
2	6	D−	0	D−
3	2	D−	0	ND
4	8	D+	2	D+
5	2	D+	1	D+
6	3	D−	0	D−
7	8	D−	0	D−
8	6	D+	2	D+
9	7	D+	1	D+
10	7	D+	3	D+

ND, Not done.

blood cells for phenotyping. Storage of the villi at 4° C for 5 days in the culture media did not alter the yield of red blood cells.

The fetal origin of three cell preparations was determined with an immunoprecipitation method that measures the individual cell fetal hemoglobin contents. All samples showed >90% fetal hemoglobin–containing cells. The corresponding maternal blood samples showed <1% fetal hemoglobin–containing cells. These results confirmed that the test samples were not being contaminated with maternal red blood cells. This contamination could potentially result in the dilution of the Rh-positive fetal cells and a reduction in the diagnostic usefulness of the immune rosette test.

The sensitivity of the immune rosette test was assessed by making artificial cell mixtures of Rh-positive cells in Rh-negative cells. As few as one Rh-positive cell in 1000 Rh-negative cells could be detected.

In the Rh-positive fetuses between one and three rosettes per high-power field were seen. A typical rosette is shown in Fig. 1. To demonstrate that the indicator cells formed rosettes around fetal cells, a fetal hemoglobin stain was performed. The indicators cells are seen rosetting around the acid-resistant cells containing fetal hemoglobin.

Table I shows the results of the chorionic villus Rh typing and the Rh phenotype testing of cord blood after delivery of the infants at term. As shown, samples weighing as little as 2 mg yielded adequate numbers of red blood cells for typing. Ten chorionic villi samples were typed; however, one of the pregnancies was electively terminated, accounting for only nine cord blood typings. There were no discrepancies between the typing results performed antenatally and those determined from the cord blood sample.

Comment

Chorionic villus sampling enables one to obtain cells for prenatal diagnosis in the first trimester. Because the villi are derived from the fertilized ovum, they reflect the genotype of the developing fetus. The development of chorionic villus sampling has thus provided early assessment of the status of developing fetuses that may be at risk for a variety of genetic disorders.[4] Although chorionic villus sampling is a safe and effective technique for early prenatal diagnosis, there may be a slightly higher risk of fetal loss than with second-trimester amniocentesis.[5]

Shortly after the institution of chorionic villus sampling, it was recognized that the capillary vessel coursing through the long axis of the villus would be an ideal source of fetal red blood cell cells. This observation was then applied to fetal Rh typing.[6] Rh typing of red blood cells from chorionic villi may serve three purposes: (1) for early fetal phenotyping in women with a history of severely affected infants with hemolytic disease of the newborn and wishing to terminate the pregnancy if the fetus is Rh positive, (2) as an alternative to cordocentesis done in the second trimester in the Rh-immunized woman with previously affected fetuses and wishing to continue with the pregnancy, and (3) in determining the need for Rh prophylaxis in Rh-negative, nonimmunized women undergoing chorionic villus sampling for genetic analysis.

The first application for fetal Rh typing is in severe immunization when the father is an apparent Rh heterozygote. Knowing the fetal Rh phenotype early in gestation may help such couples to decide whether they wish to continue the pregnancy. Before performing the procedure, one must consider that the procedure itself may lead to an increase in the antibody titer, increasing the chances of, and perhaps the severity of, intrauterine hemolysis if the fetus is Rh-positive. If severe fetal anemia develops earlier than 18 weeks of gestation after first-trimester chorionic villus sampling in a previously immunized patient, fetal intrauterine transfusion may be technically very difficult.[7]

Prospective studies about the potential risk of worsening immunization by first-trimester chorionic villus sampling have not been performed. In the report by Kanhai et al.[8] two of eight women severely immunized against Rh or Kell antigens elected to continue their

pregnancies after first-trimester chorionic villus sampling revealed the fetus to be positive for the red blood cell antigen in question. One patient whose fetus was Kell positive by chorionic villus sampling at 9 weeks required four intrauterine transfusions between weeks 23 and 31. The second patient required three intrauterine transfusions and was delivered of an Rh-positive infant at 35 weeks. Both women also underwent weekly plasmapheresis. The two babies did well. Whether the course of fetal disease was altered detrimentally by chorionic villus sampling is unknown, but neither woman produced a significant rise in antibody titers after the procedure. There are three occurrences of immune hydrops fetalis in the midtrimester of pregnancy after first trimester chorionic villus sampling on previously immunized patients (Moise, personal communication).[9] This potential risk warrants extensive patient counseling before performing early chorionic villus sampling in immunized women. Removing as small a villus sample as possible with the fewest number of passes may minimize the risk of significant fetomaternal hemorrhage.[10] Because the immune rosette method described in this report requires <10 mg of villi, the number of potentially immunizing red blood cells may be low enough to avoid such a consequence.

Many women may wish to undertake a pregnancy in spite of very severe Rh immunization. In these cases, where the father is an apparent D heterozygote, second trimester Rh phenotyping of the fetus is often desirable. At this gestational age chorionic villus sampling may be a useful alternative to cordocentesis for Rh phenotyping. Because cordocentesis is frequently performed by traversing the placenta to reach the umbilical cord insertion site, a large amount of fetomaternal bleeding may be expected to occur. Nicolini et al.[11] found that the mean estimated volume of fetomaternal hemorrhage associated with intravascular intrauterine transfusion by cordocentesis was 2.4 ml of fetal blood and furthermore that both the maternal antibody concentration and the rate of fall in fetal hematocrit was greater when the hemorrhage was >1 ml. A greater incidence of fetomaternal hemorrhage occurred when the placenta was anterior.

If chorionic villus sampling were performed as an alternative to cordocentesis, the risk of fetomaternal hemorrhage could be potentially reduced. From several reports of maternal serum α-fetoprotein rises before and after either cordocentesis or chorionic villus sampling, it appears that second and third trimester chorionic villus sampling may result in a lower incidence of significant maternal serum α-fetoprotein rises than does transplacental cordocentesis.[11-14] We have begun to examine villus samples at these later stages of gestation. Our preliminary findings on 12 samples collected from 17 to 40 weeks of gestation also indicate that these samples yield abundant fetal red blood cells for phenotyping.

Studies of the increase in maternal serum α-fetoprotein concentrations before and after first trimester chorionic villus sampling suggests that associated fetomaternal hemorrhage can occur. In some cases the volume of fetomaternal bleeding may be sufficient to cause primary isoimmunization but has yet to be proven. Therefore a consensus for giving Rh immune globulin for chorionic villus sampling has not been established. However, considering the chance for immunization and the low risk of Rh immune globulin administration prophylaxis, the use of Rh immune globulin after chorionic villus sampling is recommended by the American College of Obstetricians and Gynecologists.[7]

By typing the fetal red cells in Rh-negative women undergoing villus sampling for genetic evaluation of the fetus, it may be possible to avoid giving unnecessary Rh immune globulin prophylaxis if the fetus is Rh negative. Our results show that even early in pregnancy sufficient red blood cells can be collected for accurate Rh phenotyping.

The ability to obtain sufficient red blood cells from the smallest possible amount of chorionic villus is especially important in minimizing the risk of isoimmunization. In studies by Blakemore et al.[10] there was a positive correlation between the volume of fetal blood transferred and the weight of the chorionic villus sample. When samples weighing <10 mg were obtained, only 21 μl of fetal blood was transferred. It is unlikely this small volume of red blood cells would cause primary immunization.

The major limitation of typing fetal red blood cells from chorionic villi is the small number of red cells present. This makes standard agglutination techniques for red blood cell typing difficult to perform. Using immunofluorescence, previous investigators have shown that fetal red blood cell phenotyping from villus samples in the first trimester of pregnancy is feasible. However, a relatively large sample size was required (25 to 40 mg of villi) to obtain sufficient red blood cells for testing.[15]

To reduce the size of the chorionic villus sample needed for testing, we applied a simple immune rosette procedure for phenotyping fetal red blood cells, a procedure that could be performed by any laboratory familiar with standard immunohematologic testing. This immune rosette procedure is similar to that reported by Sebring and Polesky[16] for use in the detection of fetal maternal hemorrhage in Rh immune globulin candidates. Importantly, chorionic villus samples weighing as little as 2 mg give reliable results.

The series of patients we report were not Rh immunized. Because the rosette test will be applied to women with anti-D, we have subsequently applied the

rosette technique to three pregnancies complicated by anti-D alloimmunization. We have found that the fetal cells are typable as Rh(D) positive when anti-D is present in the patient's sample. We also prepared mixtures of Rh(D)-negative cells and Rh(D)-positive fetal cells sensitized with anti-D. In these experiments, we found that the Rh(D)-positive fetal red blood cells could be accurately typed by the rosette technique. Therefore we do not believe that the presence of anti-D will interfere with accurate Rh phenotyping of fetal red blood cells by the rosette technique.

In summary, our studies show that fetal red blood cells harvested from chorionic villus samples early in pregnancy can be accurately Rh-typed by an immune rosette method. These results will be useful in the management of pregnancies complicated by Rh immunization and may also reduce the need for unnecessary Rh immune globulin prophylaxis in Rh-negative women undergoing chorionic villus sampling.

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Infertility and pregnancy outcome in an unselected group of women with insulin-dependent diabetes mellitus

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OBJECTIVES: The null hypothesis of this study is that infertility and pregnancy outcomes in women with insulin-dependent diabetes are identical to those of nondiabetic control subjects.
STUDY DESIGN: A questionnaire survey comprising an unselected population of 18- to 49-year-old diabetic women and a comparable control group was performed. Reply rates were 94% (n = 245) and 88% (n = 253), respectively.
RESULTS: Cumulative rates of pregnancies and involuntary infertility (17%) did not differ between the two groups. Diabetic women had significantly fewer pregnancies (1.4 vs 1.7) and fewer births per pregnancy than controls, and more diabetic women were nulliparous (48% vs 38%). Half of all diabetic pregnancies were planned. Diabetic women reported that their diabetes had a negative influence on their attitude toward having children.
CONCLUSION: In insulin-dependent diabetic women the ability to conceive is normal, but diabetic women have fewer pregnancies and fewer births per pregnancy than controls. (Am J Obstet Gynecol 1992;166:1412-8.)

Key words: Epidemiology, infertility, pregnancy outcome, insulin-dependent diabetes mellitus

In the preinsulin era the occurrence of pregnancy in women with diabetes mellitus was unusual, partly because of high mortality among these patients but also because of impaired fertility in the sexually mature diabetic woman. Estimates of fertility were about 2% that of nondiabetics.[1] After the introduction of insulin therapy, life expectancy for the diabetic women increased and fertility improved. Thus Bergqvist[2] reported that from 1938 to 1952 the pregnancy rate was estimated to be 38% lower in women with diabetes than in nondiabetics. Recently it has been reported that diabetic women have the same rate of spontaneous conception and sterility as controls.[3] During the last two decades several studies have been published concerning the prognosis of pregnancy outcome in diabetic women. However, studies on fertility and outcome of pregnancies in women with diabetes have so far comprised selected groups of women, often hospitalized patients.[2-4] In a recent population study from Aberdeen only 62% of infertile women attended a hospital clinic for treatment of their infertility,[5] and in a survey on infertility from Denmark less than half the women with reduced fertility had sought medical care.[6] These results clearly indicate that population-based studies are needed if meaningful data on infertility rates are to be obtained.

The aim of this study was to investigate the epidemiologic features of infertility and pregnancy outcome in an unselected group of women with insulin-dependent diabetes mellitus. For comparison an age-comparable, unselected group of nondiabetic women was included.

Material and methods

The study was performed in the County of Funen, which has approximately 450,000 inhabitants, constituting roughly 9% of the Danish population. With regard to demographic and socioeconomic status, the population of the county is representative of the Danish population as a whole,[7] and the area is therefore suitable for epidemiologic investigation on unselected patient populations.

Patient identification. From Sept. 1, 1986, to Aug. 31, 1987, all insulin-treated patients living in the County of Funen were identified by the prescription method.[7] All women between 18 and 49 years of age with onset of diabetes before the age of 30 years and who lived in the county on July 1, 1987, were identified (N = 281) (Table I). During the period Feb. 1, 1987, to Jan. 31, 1990, all but 20 who were excluded (10 had left the county, four had died, four had mental retardation, one had non–insulin-dependent diabetes mel-

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Table I. Number of women who were identified, excluded, and gave informations on various questions

	Diabetic women (No.)	*Control women (No.)*
Identified	281	289
After primary exclusion	261	287
Answering questionnaire	245	253
After exclusion of women with diabetes that developed after their first pregnancy	201	
Who gave information on previous desire for conceiving	200	247
Who gave information on pregnancy outcome	132	186
Who gave information on fertility and who had attempted or experienced a first pregnancy	131	182
Who gave information on fertility and who had attempted or experienced a second pregnancy	83	145

litus, and one had aphasia after stroke) received a questionnaire comprising 31 main questions and 89 secondary questions. The questions related to the current survey concerned social status, time of diabetes onset, gynecologic history, present and previous desire for conceiving, number of pregnancies, and pregnancy outcome. The response rate of the 261 diabetic women entering the study was 94% (Table I). Median age of responders was 32 years (range 18 to 49 years), and median duration of diabetes 17 years (range 2 to 43 years). Of the responders 169 (69%) women were selected with a table of random numbers for more intensive investigation. Fasting C-peptide (radioimmunoassay method)[8] obtained from 163 of these 169 women was <0.20 nmol/L in 159, whereas four subjects had values between 0.22 and 0.40 nmol/L. In 44 women diabetes developed after their first pregnancy, and these women were secondarily excluded from the current study (Table I).

A group of nondiabetic women ($N = 289$), matching the diabetic women with regard to number and age distribution, was used as controls (Table I). The sample was drawn at random with a random number generator from the computerized Electronic Data Processing Department of Odense University Hospital, where all citizens in Funen County, whether healthy or ill, are registered. Two subjects were primarily excluded because of cancer. The response rate was 88%.

There were no significant differences between diabetic and control subjects with regard to marital status (75.1% diabetic and 76.7% control subjects were married), education, or occupation. In the group of 18- to 39-year-old subjects median age for the diabetic women was 28 years versus 29 years among controls ($p > 0.05$). In the 18- to 49-year-old subjects diabetic women were significantly younger than controls (median 31 years vs 33 years, respectively, $p < 0.03$).

Definitions of fertility groups. Infertility is defined as failure to conceive after attempting for >1 year. Primary infertility refers to the first pregnancy regardless of whether a pregnancy was eventually achieved. Secondary infertility refers to any subsequent pregnancy. Those women who conceived after attempting for ≤12 months are defined as having no fertility problem and are herein referred to as being fertile. Voluntary infertility refers to women never attempting pregnancy and never being pregnant. According to their history of fertility, the women were divided into three groups: those with infertility, primary or secondary; those with no fertility problem; and those with voluntary infertility. The women with primary or secondary infertility were subdivided into two groups: those with primary infertility, including those who also reported secondary infertility, and those with secondary infertility only.

Definition of planned or unplanned pregnancy. If pregnancy was desired and attempted, the pregnancy was reported as being planned, and if not, as being unplanned.

Statistics. Group comparisons concerning age were analyzed by the Mann-Whitney U test. Comparisons of fertility, number of pregnancies, and outcome of pregnancies were performed with the χ^2 test.

The study had the approval of the local ethics committee.

Results

Prevalence of infertility. One woman in the diabetic group and six women in the control group did not wish to give information concerning previous desire for pregnancy, which leaves 200 diabetics and 247 controls for investigation (Table I). Table II shows the number and prevalence rates of the different fertility groups. In tables and text the data shown in parentheses after the prevalence rates are 95% confidence limits.

There was no fertility problem in 54.5% of the diabetic women and 61.5% of the controls ($p > 0.05$). The median age of these diabetic women was 33 years, range 19 to 49 years, and for the controls 34 years, range 18 to 49 years ($p > 0.05$). A first pregnancy had been attempted or experienced by 131 diabetic women and 182 controls. The prevalence of no fertility problems

Table II. Number and prevalence rates of different fertility groups in women with insulin-dependent diabetes mellitus and in controls

	No. of subjects	*No fertility problems*	*Voluntary infertility*	*Primary and/or secondary infertility*	*Primary infertility*	*Secondary infertility only*
Diabetics						
All	200	109	69	22	17	5
%		54.5 (48-62)	34.5 (28-42)	11.0 (7-16)	8.5 (5-13)	2.5 (1-6)
AEP1	131	109		22	17	
%		83.2 (77-91)		16.8 (11-24)	13.0 (8-20)	
AEP2	83					5
%						6.0 (2-14)
Controls						
All	247	152	65	30	19	11
%		61.5 (54-67)	26.3 (21-32)	12.1 (8-17)	7.7 (5-12)	4.5 (2-7)
AEP1	182	152		30	19	
%		83.5 (77-87)		16.5 (12-23)	10.4 (7-16)	
AEP2	145					11
%						7.6 (4-13)

AEP1, Women who had attempted or experienced a first pregnancy; *AEP2*, women who had attempted or experienced a second pregnancy. Numbers in parentheses are 95% confidence limits.

in these women was 83.2% and 83.5%, respectively ($p > 0.05$).

Voluntary infertility was reported by 34.5% of diabetic women and 26.3% of controls ($p = 0.06$). The median age of these women was 24 years, range 18 to 49 years for the diabetic women and 22 years, range 18 to 49 years for the controls ($p > 0.05$). In the age group 18 to 29 years, 48 of 91 (52.7%, 47% to 69%) diabetic women were voluntarily infertile versus 59 of 99 (59.6%, 49% to 69%) controls ($p > 0.05$). Among 30- to 49-year-old diabetic women, 21 of 109 (19.3%, 12% to 28%) were voluntarily infertile versus 6 of 148 (4.1%, 1% to 9%) corresponding controls ($p < 0.005$). The proportion of married subjects in the two groups was similar (56%).

Primary or secondary infertility had been experienced by 11.0% of the diabetic population and 12.1% of the control population. Median age of these women was 31 years, range 23 to 41, for diabetic women and 37 years, range 25 to 49, for controls ($p < 0.02$). The prevalence of primary or secondary infertility in women attempting or experiencing a first pregnancy was 16.8% in diabetic women and 16.5% in controls ($p > 0.05$). Five of 131 (3.8%, 1% to 9%) diabetic women had an unresolved infertility problem versus 13 of 182 (7.1%, 4% to 12%) controls ($p > 0.05$).

Of the diabetic women 14 of 131 (10.7%, 6% to 17%) had primary infertility only and three of 131 (2.3%, 0% to 7%) had both primary and secondary infertility. Of the controls 14 of 182 (7.7%, 4% to 13%) had primary infertility only and five of 182 (2.7%, 1% to 7%) had both primary and secondary infertility ($p > 0.05$).

A second pregnancy had been attempted or experienced by 83 diabetic women and 145 controls. The prevalence of secondary infertility only among these women was 6.0% in diabetic women and 7.6% in controls ($p > 0.05$).

Unplanned or planned pregnancies and cumulative rate of pregnancies. Among diabetic women and controls significantly more pregnancies were unplanned in the fertile group of women than in the infertile group, 114 of 219 (52.1%, 45% to 59%) versus eight of 42 (19.0%, 9% to 34%) for diabetic women ($p < 0.001$) and 201 of 365 (55.1%, 53% to 64%) versus nine of 44 (20.5%, 10% to 35%) for controls ($p < 0.0005$). There were no significant differences between diabetic women and controls when the same fertility groups are compared. The pregnancies took place between 1959 and 1989 for the diabetic women and controls. From 1959 to 1969 13 of 33 (39.4%, 23% to 58%) of the diabetic pregnancies were planned. The value from 1970 to 1979 was 56 of 113 (49.6%, 40% to 60%) and from 1980 to 1989 70 of 122 (57.4%, 49% to 67%). Thus the diabetic women had 139 of 261 (53.3%, 48% to 60%) planned pregnancies versus 199 of 409 (48.7%, 45% to 55%) in controls. The cumulative rate of these pregnancies (Fig. 1) did not differ significantly between diabetic women and controls. Thus after 1 year the fertility rate was 85% for the diabetic group and 88% for the controls. When the cumulative rate of pregnancies of the fertile diabetic women is compared with that of the fertile controls, no difference was found ($p > 0.05$).

Pregnancy rate and outcome of pregnancies. All diabetic women gave information on the outcome of pregnancies, whereas two of the controls declined. The median maternal age for the first pregnancy was 23 years, range 16 to 32 years, for the diabetic women and 23 years, range 15 to 40 years, for the controls. All the diabetic pregnancies and all but two of the control pregnancies took place when the women were between 18 and 39 years old.

Table III shows the pregnancy rate and pregnancy outcome for all pregnant women and for each fertility

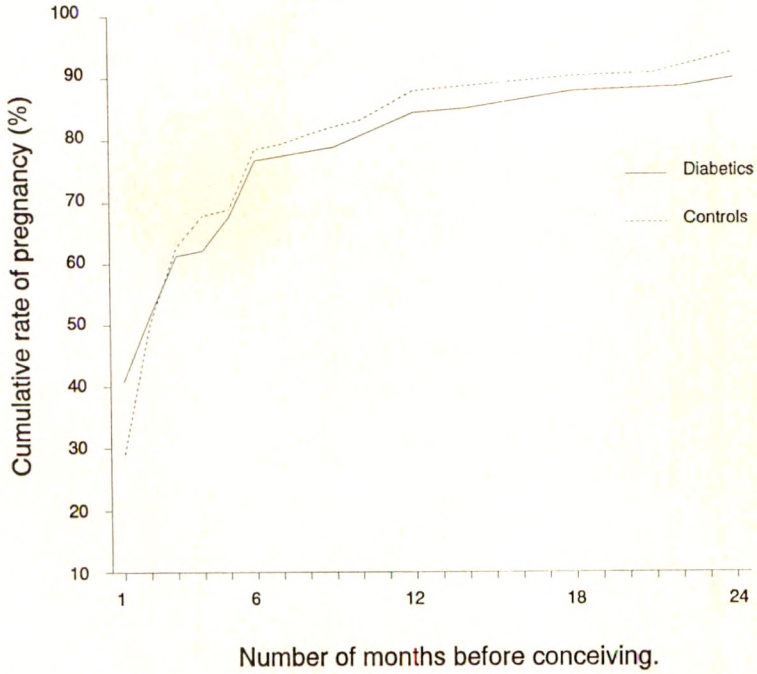


Fig. 1. Cumulative rate of pregnancy (time to conception) over months for 139 diabetic pregnancies and 199 control pregnancies (1959 to 1989).

group. The fertile and infertile diabetic women were significantly younger than corresponding controls (median 33 years, range 19 to 49 years versus 35 years, range 18 to 49 years, respectively) ($p < 0.003$). After the number of pregnancies for this age difference is adjusted, there is no significant difference in the number of pregnancies per fertile plus infertile woman in the two groups. For all the diabetic and control subjects surveyed, the pregnancy rate was significantly lower in the diabetic group (mean 1.3 and adjusted for age mean 1.4) compared with the control (mean 1.7) ($p < 0.01$ for both comparisons). Overall birth rate was lower in diabetic women compared with controls (69.5% vs 76.5%, $p < 0.05$). The infertile diabetic women had a lower birth rate ($p < 0.05$) and a higher spontaneous abortion rate ($p < 0.005$) compared with fertile diabetic women.

Cesarean section rate. The overall rate of cesarean sections was 82 of 182 (45.1%, 38% to 53%) in diabetic women and 39 of 322 (12.1%, 9% to 17%) in controls ($p < 0.001$). The rate in infertile diabetic women was 11 of 23 (47.8%, 27% to 69%) versus eight of 33 (24.2%, 11% to 42%) in controls ($p = 0.07$). For the fertile diabetic women the rate was 71 of 158 (44.9%, 37% to 52%) and for the fertile controls 31 of 277 (11.2%, 8% to 16%) ($p < 0.001$).

Number of births per woman. Significantly more diabetic women had no children, 96 of 201 (47.8%, 41% to 56%) compared with controls 94 of 251 (37.5%, 32% to 47%) ($p < 0.03$) and significantly fewer diabetic women had two or more children, 58 of 201 (28.9%, 23% to 36%) versus 118 of 251 (47.0%, 41% to 54%) for controls ($p < 0.01$).

Influence of the diabetes on the attitude toward having children. The diabetic women were questioned whether their diabetes had an influence on their attitude toward having children. Of the 197 who answered the question, 111 (56.3%, 47% to 62%) women gave a positive answer. Seventy-six (38.6%, 31% to 45%) were concerned about possible complications during pregnancy and delivery, 64 (32.5%, 26% to 39%) were concerned about an aggravation of diabetic control during the pregnancy, 57 (28.9%, 22% to 35%) were concerned about the risk that diabetes might develop in the child, 26 (13.2%, 9% to 19%) reported that the strain of daily diabetes care had a negative influence on their attitude toward having children, and 26 (13.2%, 9% to 19%) mentioned other reasons. In the voluntary infertility group, significantly more women, 47 of 67 (70.1%, 58% to 81%) gave a positive answer to the question compared with the rest, 64 of 130 (49.2%, 40% to 58%) ($p < 0.03$).

Comment

In general, the presence of infertility is considered psychologically distressing by most women, and much attention has therefore been directed toward identification and treatment of this problem. In the diabetic population infertility may add to the social strain of daily diabetic care and to potential diabetic complications. In these women early discovery of an infertility problem is especially important, because advanced ma-

Table III. Pregnancy outcome in women with and without infertility problems, for diabetic women and controls

	Diabetic women						*Controls*					
	All (n = 132)*		*Infertile (n = 22)*		*Fertile (n = 109)*		*All (n = 186†)*		*Infertile (n = 30)*		*Fertile (n = 152)*	
Outcome	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
Birth	182‡	69.5 (64-76)	23§	54.8 (39-70)	158	72.1 (66-78)	322	76.5 (73-81)	33	75.0 (60-87)	277	75.9 (70-85)
Spontaneous abortion	39	14.9 (11-19)	14‖	33.3 (20-50)	25	11.4 (7-16)	44	10.5 (7-13)	7	15.9 (7-30)	37	10.1 (7-15)
Induced abortion	38	14.5 (10-19)	3	7.1 (2-20)	35	16.0 (11-21)	51	12.1 (9-16)	3	6.8 (1-19)	48	13.2 (10-18)
Ectopic pregnancies	3	1.1 (0-3)	2	4.8 (1-16)	1	0.5 (0-2)	4	1.0 (0-2)	1	2.2 (0-12)	3	0.8 (0-2)
All pregnancies												
Total No. of pregnancies	262		42		219		421		44		365	
Mean	2.0		1.9		2.0		2.3		1.5		2.4	
Median	2		2		2		2		2		2	
Range	0-7		0-5		1-7		0-5		0-4		1-5	

Values in parentheses are 95% confidence limits.

*One woman gave no information on the time before conception; she had one birth.

†Four women gave no information on the time before conception, they had 12 births.

‡Significantly different from all controls, $p < 0.05$.

§Significantly different from fertile diabetics, $p < 0.05$.

‖Significantly different from fertile diabetics, $p < 0.005$.

ternal age and diabetic complications may carry an increased pregnancy risk.[9-11]

Previous studies on the frequency of infertility in diabetic women are few[1-4] and seem to comprise only selected patients. To our knowledge the current study is the first published survey on infertility and pregnancy outcome in an unselected population of diabetic women.

In a previous study[7] in which a similar patient identification method was used, the estimated completion of the study material was 98%. Our response rate of 94% in the diabetic population and 88% in the control population is higher than obtained in most other questionnaire studies on gynecologic topics.[5, 12, 13] Thus our methods concerning population identification and data sampling seem sufficient.

The definition of infertility is arbitrary. Some authors define infertility as failure to conceive after 2 years without contraception,[5, 13] whereas most demand only 1 year, and the proportion of couples to whom this applies is between 10% and 28%.[13-17] The infertility rate of 17% as observed by us in the control group is comparable to these results and probably represents the size of the infertility problem in the general population.

Our results show that the overall rate of involuntary infertility in diabetic women of 17% is virtually identical to that of controls. This finding seems surprising because menstrual disturbances in these women occur with a frequency twice that of the controls (Kjaer K, et al. Unpublished data). We have no explanation for these conflicting results. Recent studies in unselected diabetic populations are not available, but in selected diabetic patients an infertility rate of 21% has been reported from a pregnancy clinic.[4]

Diabetic women and controls also have the same cumulative rate of pregnancy, which suggests that the time to conceive is similar in the two groups. As also observed in other studies,[5, 13] most of our infertile diabetic women and infertile controls had primary infertility, and more than half of these women eventually became pregnant. There was no trend toward a lower frequency of pregnancies in infertile diabetic women compared with infertile controls. Infertile diabetic women were significantly younger than infertile controls, which may indicate that infertility as a clinical problem may occur at an earlier stage in diabetic women compared with nondiabetics.

Our investigation has shown that there was a trend toward more women being voluntarily infertile in the diabetic population than among controls. Further, voluntary infertility occurs with similar frequency in younger diabetics compared with controls but seems to be increased among older diabetic women. This finding may partly be attributed to the professional advice given by diabetologists and gynecologists who call for caution when diabetic women >30 years of age want to become pregnant. Another possibility is that the diabetic women >30 years have reached the age of re-

production at a time when physicians' attitudes toward diabetic pregnancy were considerably more negative than in recent years, and therefore these women may have been convinced of the inadvisability of pregnancy early in their reproductive years. The trend toward more diabetic women being voluntarily infertile may also be due to the observation that the presence of diabetes has a negative influence on their attitude toward having children.

It is generally accepted that poor metabolic control before and during the first weeks of pregnancy in diabetic women is associated with a substantial increased risk of spontaneous abortion and congenital defects. Such complications may be reduced to levels roughly equal to that of the general population if optimal metabolic control can be obtained.[18-22] Accordingly, modern diabetic care involves meticulous planning of pregnancies and the application of intensive metabolic monitoring and treatment to ensure the best possible blood glucose regulation in the individual patient. The present investigation has shown that 47% of all pregnancies in diabetic women in the period 1959 to 1989 was unplanned, and this value seems also to apply to the general population. Although the prevalence of unplanned pregnancies was highest in the first decade (1959 to 1969, 60%), when the importance of birth planning was not widely accepted, the rate of 43% during the last decade (1979 to 1989) is still unacceptably high. In the view of the authors this may raise serious doubts whether the therapeutic principles in diabetic women of fertile age are fully implemented in practice. Funen County has had a center for pregnant diabetic women with a diabetologist and an obstetrician since 1968; therefore this lack of planning may apply also to other centers in the Western world.

The 1.7 per woman rate of pregnancy in our control group is lower than that of 2.3 observed in a previous Danish study on 709 25- to 45-year-old women surveyed in 1979.[13] A deliberate choice to limit family size is probably responsible for most of the decline. Their induced abortion rate of 7% was somewhat lower compared with ours (12%). Because the former study was carried out about a decade before ours, this discrepancy is probably explained by the different access to legalized abortion (legalized in Denmark in 1973).

In diabetic women, the number of pregnancies is reduced compared with controls, in spite of the fact that the two groups were comparable with regard to involuntary infertility. As discussed previously, this observation is likely to be caused by the attitude on the part of both patient and therapist toward pregnancy in diabetic women. As expected, pregnancy outcome in diabetic women is characterized by fewer births per pregnancy compared with controls.

Our cesarean section rate of 45% among diabetic deliveries is comparable to that of 53% to 54% from other Danish centers.[23, 24] Although specific data on indication for cesarean section in our study groups are not obtainable, there is no doubt that the much higher rate in diabetic women is due to the increased perinatal risks in these patients.[25]

Because legalized abortion was introduced fairly early in Denmark compared with most other countries, meaningful comparisons of the rate of spontaneous or induced abortion are not possible. In our study spontaneous abortion was moderately increased in diabetics (15%) compared with controls (11%), which may indicate that optimal metabolic regulation before conception and during the first weeks of pregnancy had not been achieved. Another explanation could be the possibility of recall bias, with diabetic women being more aware of early spontaneous abortion than nondiabetic women because of their medical care. Our spontaneous abortion rate of 15% among diabetic women is comparable to that of 14% from Rigshospitalet, Denmark, from the years 1967 to 1986.[26]

In conclusion, fertility in diabetic women seems to be similar to that of the general population. Although optimal diabetic care can reduce the risks and complications of pregnancy and birth to normal levels, the majority of diabetic women still harbors a negative attitude toward having children because of their disease. This attitude seems to result in fewer pregnancies per diabetic woman and a higher proportion of diabetic women abstaining from having children compared with controls. Moreover, a medically unacceptable proportion of diabetic pregnancies is unplanned. There appears to be a need for intensifying the educational and therapeutic efforts in diabetic women of fertile age.

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Effects of mefenamic acid on menstrual hemostasis in essential menorrhagia

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Prostaglandin synthesis inhibitors decrease menstrual blood loss by 30% to 50% in patients with essential menorrhagia. To obtain insight into their mechanism of action, we measured menstrual blood loss in menorrhagic women, who were receiving mefenamic acid (500 mg, three times daily) (n = 6) or placebo (n = 5) in a double-blind way. In addition we studied the morphology of early menstrual hemostasis. The subjects' uteri were extirpated in the first 24 hours of menstruation, and light and electron microscopy were used to perform morphologic and morphometric studies. In the group treated with mefenamic acid mean menstrual blood loss was decreased by 40%. In uteri of the women treated with mefenamic acid hemostatic plugs were further transformed, and fewer vessels without a plug were observed than in uteri of the group receiving placebo. These data suggest that mefenamic acid may act through an improvement of platelet aggregation and degranulation and through increased vasoconstriction. (Am J Obstet Gynecol 1992;166:1419-28.)

Key words: Menorrhagia, mefenamic acid, endometrium, morphometry, hemostasis.

Prostaglandin synthesis inhibitors are currently the treatment of first choice in essential menorrhagia (i.e., a measured menstrual blood loss of more than 80 ml from a normal secretory endometrium after a normal ovulatory cycle). They inhibit cyclooxygenase, the enzyme that metabolizes arachidonic acid toward unstable endoperoxides. These endoperoxides are converted further toward prostaglandins. Prostaglandin synthesis inhibitors diminish menstrual blood loss by 30% to 50% and have a low incidence of side effects.[1, 2] Mefenamic acid is the best-studied prostaglandin synthesis inhibitor in essential menorrhagia.

In the endometrium of menorrhagic women, an increased fibrinolysis[3] and a shift in uterine prostaglandin metabolism towards vasodilating and platelet aggregation–inhibiting prostaglandins (prostaglandin E_2 and I_2) have been observed.[4-7] More binding sites for prostaglandin E_2 were also found in the myometrium of menorrhagic women.[8] Treatment of menorrhagic women with mefenamic acid decreased endometrial prostaglandin E_2 concentrations and myometrial prostaglandin E_2 binding sites.[9, 10]

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6/1/34906

Despite this knowledge, the mechanism of action of prostaglandin synthesis inhibitors in essential menorrhagia is not yet understood, though an effect on menstrual hemostasis is assumed.

It has been previously demonstrated that hemostasis in human skin wounds takes place through platelet adhesion to the subendothelium of damaged vessels followed by platelet aggregation and degranulation. Finally fibrin fibers are formed between the platelet remnants to preserve a stable, fully transformed, occlusive plug.[11] Hemostasis in menstrual endometrium was also studied, and differences were found compared with that in skin wounds. In the endometrium, damaged vessel walls often occurred without any hemostatic reaction, and the relatively small hemostatic plugs seldom occlude the vessel completely as they usually do in skin wounds. Only during the first 20 hours of menstruation were hemostatic plugs found. Thereafter hemostasis is probably provided by vasoconstriction.[12]

In a recent study[13] uteri of menstruating menorrhagic women were found to contain two forms of hemostatic plugs that had never before been observed in normally menstruating women.[12] First, huge fragile plugs with large protrusions into the extravascular space were observed. This condition was evidently the result of a decreased platelet aggregation and degranulation, which may reflect increased prostaglandin I_2 and E_2 concentrations or an unknown local anticoagulant. Second, otherwise well-transformed plugs often demonstrated extensive recanalization, which may reflect increased fibrinolysis. We also found that increased menstrual blood loss and, hence, essential menorrhagia

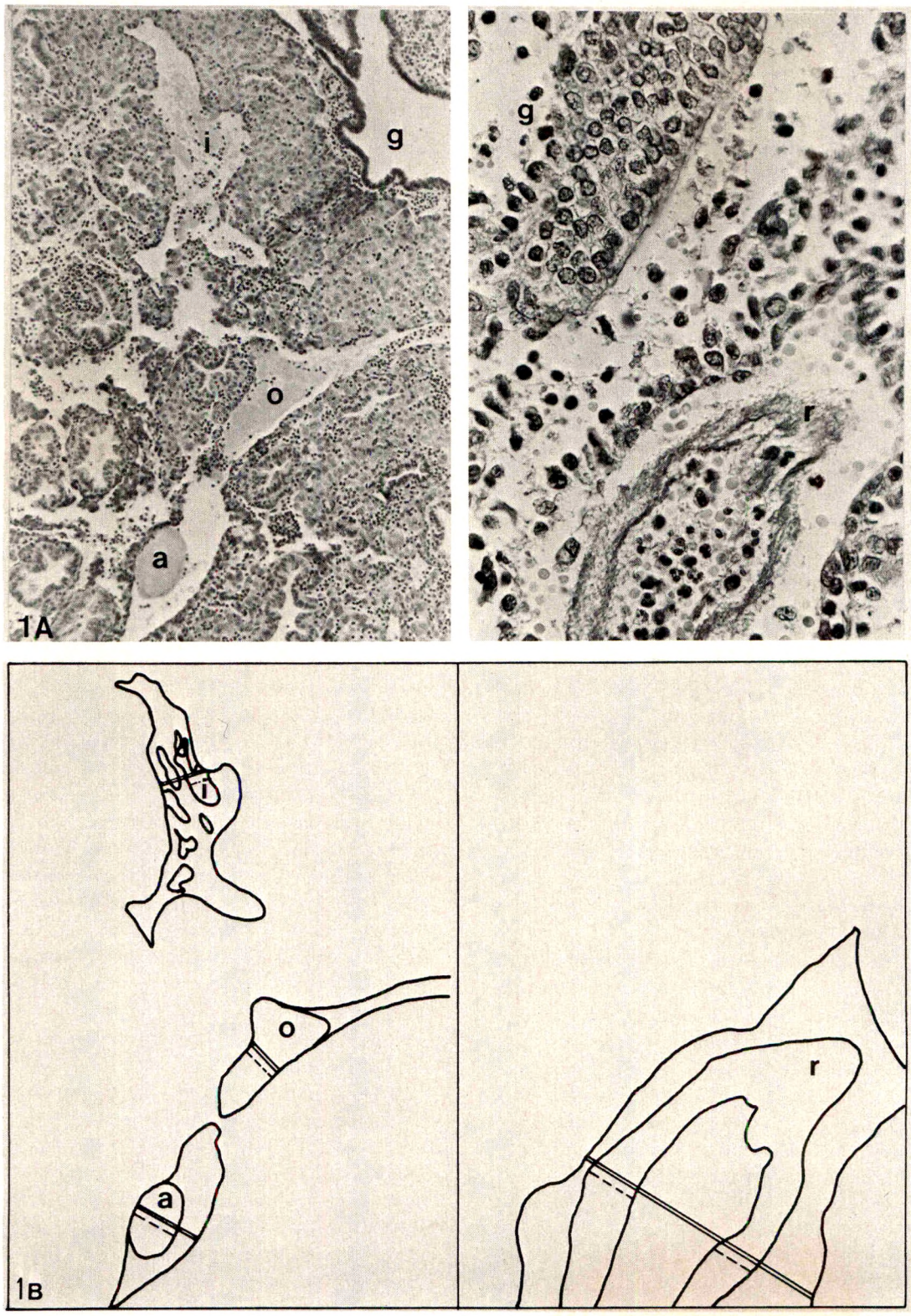


Fig. 1. Light micrographs (**1A,** *upper panels*) and corresponding line drawings (**1B,** *lower panels*) from paraffin sections stained according to Martius scarlet blue[13] showing various plug types and diameters measured. Diameters were measured at smallest width. *Solid* and *dotted lines* represent diameters of vessels and plugs. Relative occluded vessel diameter was calculated by dividing diameter of plug by diameter of vessel. *a,* Nonocclusive plug, smaller than occlusive, may be adherent to vessel wall (as pictured) or in middle of vessel (not pictured); *g,* gland; *i,* indifferent plugs (plugs not fitting other descriptions); *o,* occlusive plug, hemostatic plug occluding vessel; *r,* recanalized plug, peripheral platelet and fibrin deposition representing a former occlusive hemostatic plug that is recanalized. (Original magnification: *left,* ×90; *right,* ×358.)

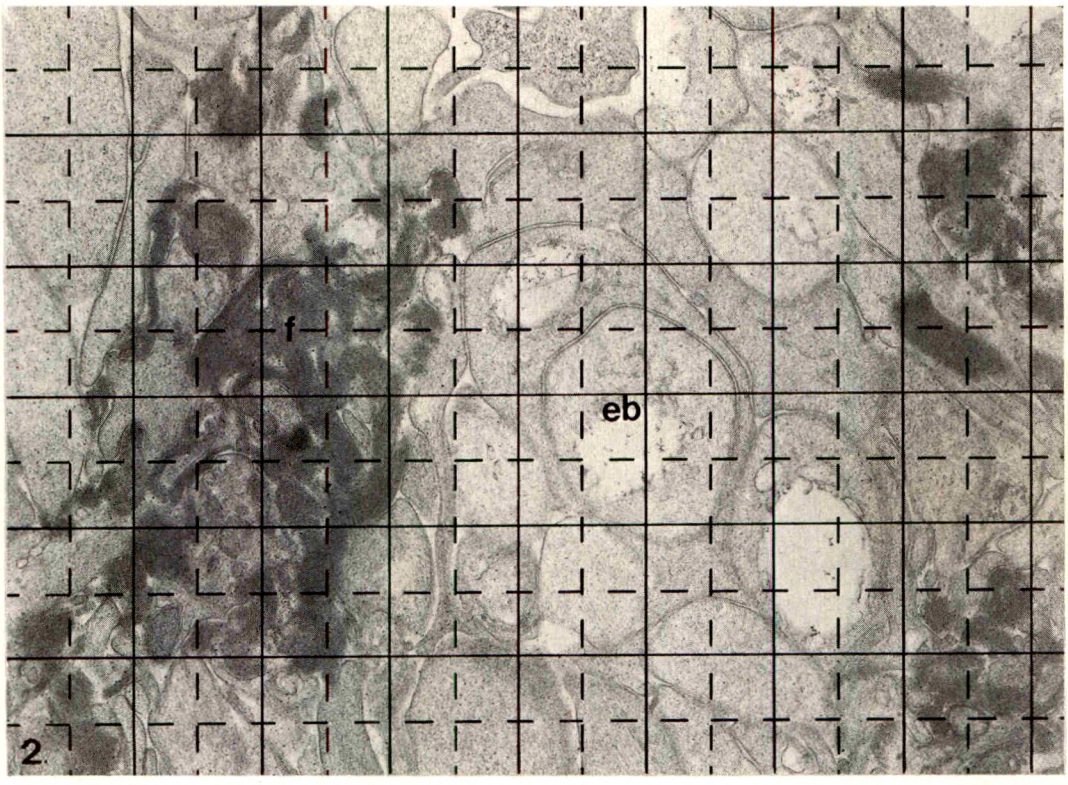


Fig. 2. Morphometric method used to assess volume fractions of empty balloons and fibrin fibers. Electron micrograph showing part of a hemostatic plug, mainly consisting of empty balloons *(eb)* and fibrin fibers *(f)*. *Solid* and *dotted lines* were used to measure volume fractions of empty balloons and fibrin fibers, respectively. We used the so-called point-hit method of counting line intersections overlying empty balloons and fibrin fibers. Numbers were expressed as percentages of total number of intersections counted. (Original magnification ×20,160.)

were positively correlated with the number of hemostatic plugs.

The present study was designed to investigate the effect of prostaglandin synthesis inhibitors on menstrual hemostasis in essential menorrhagia.

Material and methods

Patients scheduled for hysterectomy because of subjective menorrhagia were asked to participate in the trial and informed consent was obtained. Only women who met the following criteria entered the trial: younger than 45 years of age; measured menstrual blood loss more than 80 ml; regular menstrual cycle; no intrauterine device; no use of nonsteroidal antiinflammatory drugs or other medication that could interfere with hemostasis; no contraindications against the use of nonsteroidal antiinflammatory drugs, such as liver or kidney function impairment, stomach ulcers, or asthmatic bronchitis; and no use of hormonal medication. The women had normal-sized uteri as assessed by bimanual palpation.

The time schedule of the trial was as follows: menstrual blood loss was measured during one menstruation (control period) with the previously described alkaline hematin method.[14] If blood loss exceeded 80 ml, patients entered the trial. Five days before the expected next menstruation (study period) they started to use mefenamic acid (Ponstan) 500 mg or a placebo three times daily. Neither the patient nor the treating physician was aware of the nature of the administered drug. The sequence of placebo and tablets containing mefenamic acid was determined according to a random list kept at the Pharmacy of the University Hospital Utrecht. Patients continued to use tablets until menstrual bleeding had arrested. Menstrual blood loss was then measured again. Five days before the expected next menstruation, patients started to use the same tablets as during the study period. Vaginal or abdominal hysterectomy was performed in the patient under general anesthesia during the first 24 hours of menstruation. Basal body temperature charts were kept and progesterone levels were assessed with radioimmunoassay in the midluteal phase of the preoperative cycle and at the time of operation.[15] Plasma levels of mefenamic acid were assessed with a high-pressure liquid chromatographic method during the study period and at the time of operation.

The study was approved by the committee of research on human beings at the University Hospital Utrecht.

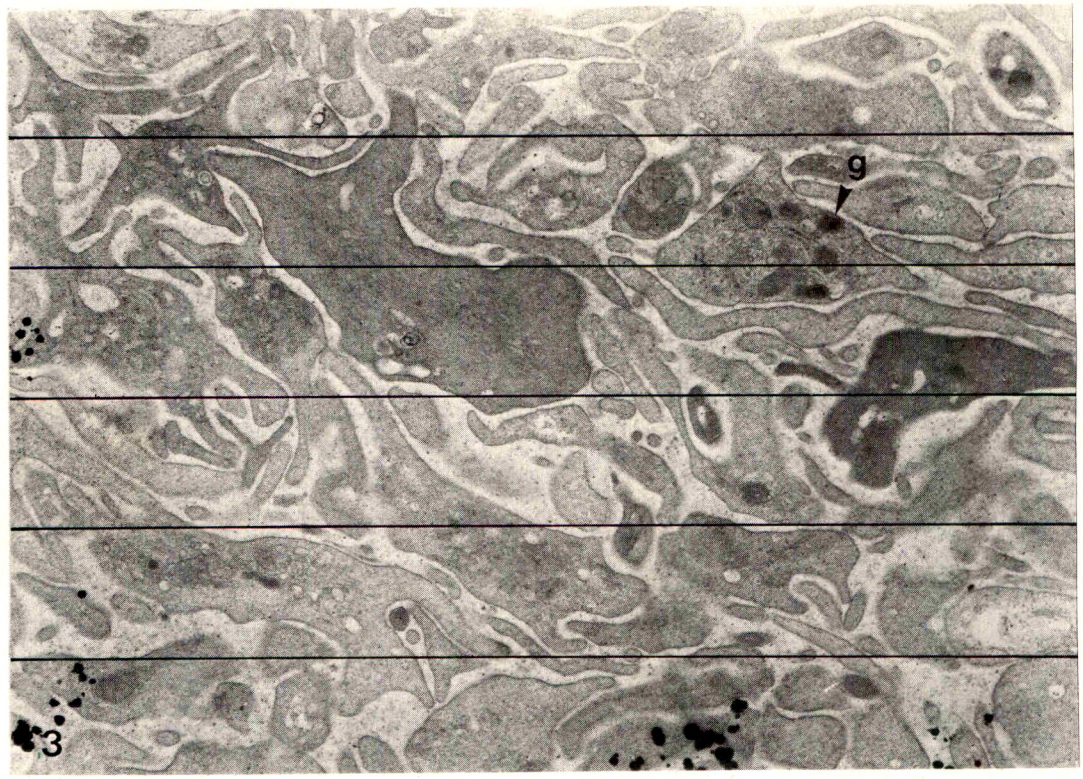


Fig. 3. Morphometric method used to assess platelet membrane surface area and granule numeric density. Electron micrograph showing part of hemostatic plug consisting of granulated and degranulated platelets. Numbers of intersections between platelet membranes and superimposed lines were counted according to linear intercept method. Platelet membrane surface area was calculated by dividing number of intersections times two by total length of superimposed line. Granules *(g)* were counted separately and granule numeric density was calculated by dividing number of granules by section thickness (70 nm). A calibration replica was used to correct for magnification. (Original magnification ×16,800.)

The placebo group was described as part of the study group in our previous study on menstrual hemostasis in essential menorrhagia.[13]

Tissue processing and morphometry. Tissue processing and morphometric methods using light and transmission electron microscopy were described in our previous study.[13] Morphometric analysis was performed on light microscopical sections with respect to endometrial height, number and diameter of endometrial vessels with or without a hemostatic plug, and size and form of hemostatic plugs. Four characteristic forms were distinguished: recanalized, occlusive, nonocclusive and indifferent (see Fig. 1). With electron microscopy, the transformation of the hemostatic plug was studied: volume fractions of fibrin and empty balloons (see Fig. 2) and membrane surface area and granule density (see Fig. 3) were calculated.[16] The extravascular extension of hemostatic plugs was also studied with electron microscopy. Morphometric parameters were expressed for each uterus. The double-blind codes were broken only after morphometric determinations had been finished.

Statistical analysis. Because not all variables were normally distributed, the Wilcoxon rank sum test was used to study differences between the two groups. The Spearman rank correlation coefficient was used to study correlations between the difference in menstrual blood loss of the mefenamic acid group (menstrual blood loss in the control cycle minus menstrual blood loss in the study cycle) and morphometric data.

Results

Patients. After 4 years and with the cooperation of many patients and gynecologists a sufficient number of uteri for our study were extirpated in the first 24 hours of menstruation.

Nineteen patients entered the trial. Seven patients dropped out from the trial after the study cycle or at the time of operation for the following reasons: Four patients postponed hysterectomy or wanted only a planned hysterectomy; one was found to have a large leiofibromatous uterus at operation; one was already in a postmenstrual phase at operation; and one was in a premenstrual phase at operation. The one discontin-

Table I. Patient characteristics

Characteristic	*Placebo group (n = 5)*		*Mefenamic acid group (n = 6)*	
	Mean	*SD*	*Mean*	*SD*
Age (yr)	39.4	3.0	39.8	3.6
Plasma progesterone levels (nmol/L)				
Midluteal	40.6	19.8	27.3	14.9
At operation	3.8	3.5	4.7	3.0
Biphasic basal body temperature chart	+	—	+	—
Menstrual hours*	13.4	5.8	10.5	5.2
Plasma levels of mefenamic acid (μg/ml)				
Medicated cycle	—	—	4.39	3.09
At operation	—	—	2.69	4.44

*Interval between onset of menstruation and operation (hours).
There were no statistically significant differences.

Table II. Menstrual blood loss (milliliters)

Placebo (n = 5)		*Mefenamic acid (n = 6)*	
Control period	*Study period*	*Control period*	*Study period*
106	92	81	36
124	183	86	56
130	207	97	62
182	286	105	78
217	178	126	92
		154	68
Mean 151	189	108*	65*
SD 46	69	27	19

*$p = 0.01$. Other differences were not significant.

uation was in a patient who stopped medication because of severe skin rash and itching. Her tablets contained mefenamic acid.

Eventually 11 uteri were available for examination, six from women in the mefenamic acid group and five from the placebo group. Only a few side effects were reported spontaneously. In the placebo group one patient mentioned headache and another itching. In the mefenamic acid group one patient mentioned stomach pains and, apart from the patient who discontinued the trial because of itching, another patient also complained of itching. No additional side effects were mentioned when the patients were questioned.

Characteristics of the patients whose uteri were studied are shown in Table I. Progesterone levels measured at the midluteal phase and at the time of operation, together with the biphasic basal body temperature charts, indicate that preoperative cycles were ovulatory. Mefenamic acid levels during the treatment cycle and at operation show great variation but prove that all patients did take their tablets.

Menstrual blood loss. Data on menstrual blood loss are shown in Table II. Mean menstrual blood loss in the control period did not differ between both groups ($p = 0.09$). In the mefenamic acid group, all menstrual blood losses decreased during the study period and mean menstrual blood loss dropped by 40% ($p = 0.01$). All patients but one had menstrual blood loss in the normal range when treated with mefenamic acid. Mean menstrual blood loss in the control and the study period did not differ in the placebo group ($p = 0.46$).

Morphologic findings.

Morphometric data. Morphometric data are presented in Table III.

Aspect of the endometrium. Macroscopically, all 11 uteri had dark red, blood-engorged areas at hysterectomy. Microscopically, all uteri had endometria partly covered with epithelium, indicating that menstruation was still in an early phase at the time of hysterectomy. Endometrial height did not differ between the two groups, nor was endometrial height correlated with the interval between the onset of menstruation and the time of operation in the two groups.

Number of vessels with and without a plug. The total number of vessels seen in light microscopy, venules as well as arterioles, was significantly reduced in the mefenamic acid group compared with the placebo group ($p = 0.03$), which can be attributed to vessels without a plug ($p = 0.02$) because it is not true for vessels with a plug ($p = 0.60$). A possible explanation may be a

Table III. Morphometric data of endometrium, endometrial vessels, and hemostatic plugs in menstruating uteri of menorrhagic women receiving placebo and mefenamic acid

	Assessment technique	Placebo (n = 5)			Mefenamic acid (n = 6)		
		Mean	SD	Range	Mean	SD	Range
Endometrial height (mm)	LM	2.4	1.0	1.3-3.6	3.5	1.4	1.8-5.5
Endometrial vessels							
Total vessel profiles (No.)	LM	564	118	445-721	384	116	282-611*
With hemostatic plug (No.)	LM	106	50	52-178	118	38	69-161
With disrupted endothelial lining (%)	EM	46	16	27-61	63	18	41-91
With disrupted endothelial lining and with plug (%)	EM	62	34	5-91	70	19	38-93
Vessel diameter							
Diameter (μm)	LM	41	8	32-53	51	8	41-62
Arterioles†		31	8	23-44	50	15	30-72*
Diameter with plug (μm)	LM	67	15	44-85	68	3	64-71
Diameter without plug (μm)	LM	34	29	31-38	42	9	32-57
Arterioles†		27	7	20-39	45	12	33-67*
Hemostatic plugs							
Size of plug							
Area occluded by plug (%)	LM	54	10	43-67	61	10	46-75
Venules†		50	10	37-63	62	10	48-76*
Extravascular plugs (%)	EM	26	16	0-42	26	17	0-50
Form of plug							
Recanalized plugs (No.)	LM	11	22	0-51	16	12	0-33
Arterioles†		1	3	0-7	5	4	0-9*
Occlusive plugs (No.)	LM	38	19	24-70	40	22	15-77
Nonocclusive plugs (No.)	LM	53	28	28-92	54	28	27-105
Indifferent plugs (No.)	LM	4	5	0-10	8	5	3-16
Transformation of plug							
Fractional volume of fibrin (%)	EM	18	4	11-21	22	9	15-35
Fractional volume of empty balloons (%)	EM	8	4	3-13	9	6	3-21
Membrane density (surface area of platelet membranes in $\mu m^2/\mu m^3$)	EM	3.4	0.6	2.6-4.1	3.2	0.7	2.4-4.0
Granules (No./μm^3)	EM	2.3	0.7	1.4-3.1	1.6	0.6	0.9-2.7

LM, Assessed in light microscopy; *EM*, assessed in electron microscopy. In light microscopy, all numeric data are expressed per 125 section fields (except for the endometrial height, which was expressed per 10 sections) at a magnification of ×25; in electron microscopy, per 10 to 15 ultrathin sections or 30 micrographs at ×7000 initial magnification.

*Significant p values (0.02 to 0.05).

†Data on arterioles and venules are given only if a statistically significant difference existed. In the placebo group, as well as in the mefenamic acid group, the ratio between venules and arterioles was 4:1.

more extensive vasoconstriction of vessels in the mefenamic acid group compared with that of the placebo group. Consequently, small constricted vessels will have escaped notice. Another possible explanation, a reduced vessel number in the mefenamic acid group, seems less probable, because mefenamic acid given 5 days before the expected menstruation is unlikely to reduce the number of vessels in secretory endometrium.

Menstrual blood loss in women in the placebo group correlated positively with the number of hemostatic plugs, both occlusive and nonocclusive, but not with other morphometric data.[13] This correlation was not seen in the mefenamic acid group.

Disruption of endothelium with and without a hemostatic plug was observed in the mefenamic acid and the placebo group to the same extent. Increased plugging of such vessel holes was thus not the explanation for the decreased blood loss in the mefenamic acid group.

Vessel diameter. There were no differences in the diameters of the total number of vessels between the mefenamic acid and placebo group. More specifically, this held true for venules but not for arterioles, which were significantly larger in diameter in the mefenamic acid group compared with the placebo group (p = 0.05). This difference between the total population of vessels and the population of arterioles can be understood if one realizes that in both groups more venules were counted than arterioles, with a ratio of 1:4 (see Table III). This finding of more diluted arterioles in the mefenamic acid group is in apparent contrast to the ob-

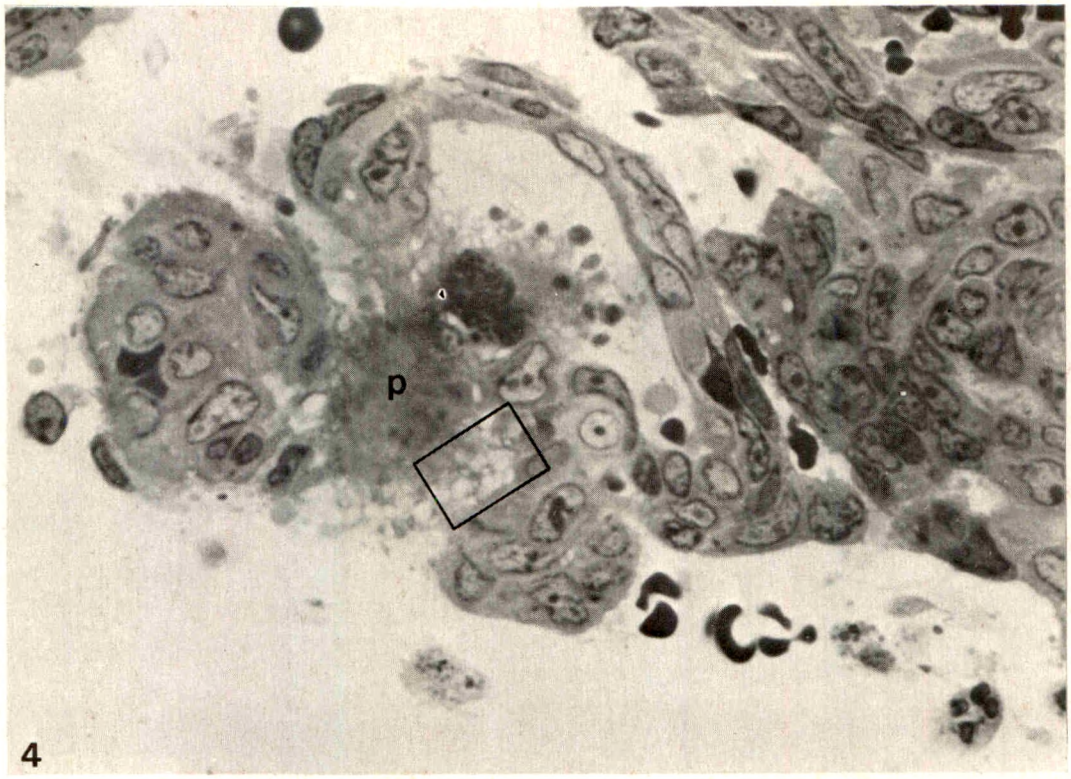


Fig. 4. Light micrograph of epon section showing small hemostatic plug *(p)*, with extravascular extension from patient receiving mefenamic acid. (Original magnification ×2163.)

servations above, where we concluded that vessels in the mefenamic acid group, venules as well as arterioles, probably were more contracted compared with the placebo group. This difference can be understood if one assumes that because of the greater amount of contracting cells in their vessel wall small arterioles are more often contracted than small venules and hence less noticed. Consequently the arterioles in the mefenamic acid group will be fewer and larger.

Size of plugs. Plugs in both groups varied in size from small to huge and loose ones. In the mefenamic acid group, fewer huge plugs were observed than in the placebo group. Plugs extending into the extravascular space were observed in both groups (see Figs. 4 and 5), but the extravascular extensions were smaller in the mefenamic acid group. This difference could not be confirmed with electron microscopic morphometric analysis (see Table III), probably because of the limited number of cross sections available. The area occluded by hemostatic plugs in venules and small arterioles was greater in the mefenamic acid group than in the placebo group ($p = 0.05$).

Form of plugs. All four characteristic plug types, recanalized, occlusive, nonocclusive, and indifferent, were observed in both groups. Recanalized plugs were observed significantly more often in arterioles of the mefenamic acid group ($p = 0.05$). However, this observation is of limited value inasmuch as the total number of recanalized plugs in arterioles is small (seven in the placebo group and nine in the mefenamic acid group). There were no significant differences in other forms of hemostatic plugs. As described earlier,[13] menstrual blood loss in the placebo group was positively correlated with the number of both occlusive and nonocclusive plugs.

Transformation of the plug. Direct observation showed that plugs of women treated with mefenamic acid seemed further transformed than those of women treated with placebo; platelets were more firmly interdigitated and more degranulated and more empty balloons and fibrin fibers were noticed (see Figs. 6 and 7). The transformation of the hemostatic plugs was also studied morphometrically at the electron microscopic level. Good correlation was observed between the decrease in menstrual blood loss during use of mefenamic acid and combined parameters of further transformation of the plug ($p < 0.025$).

Comment

The reduction of menstrual blood loss by prostaglandin synthesis inhibitors is well known but poorly understood. In this study, we describe the effect of one

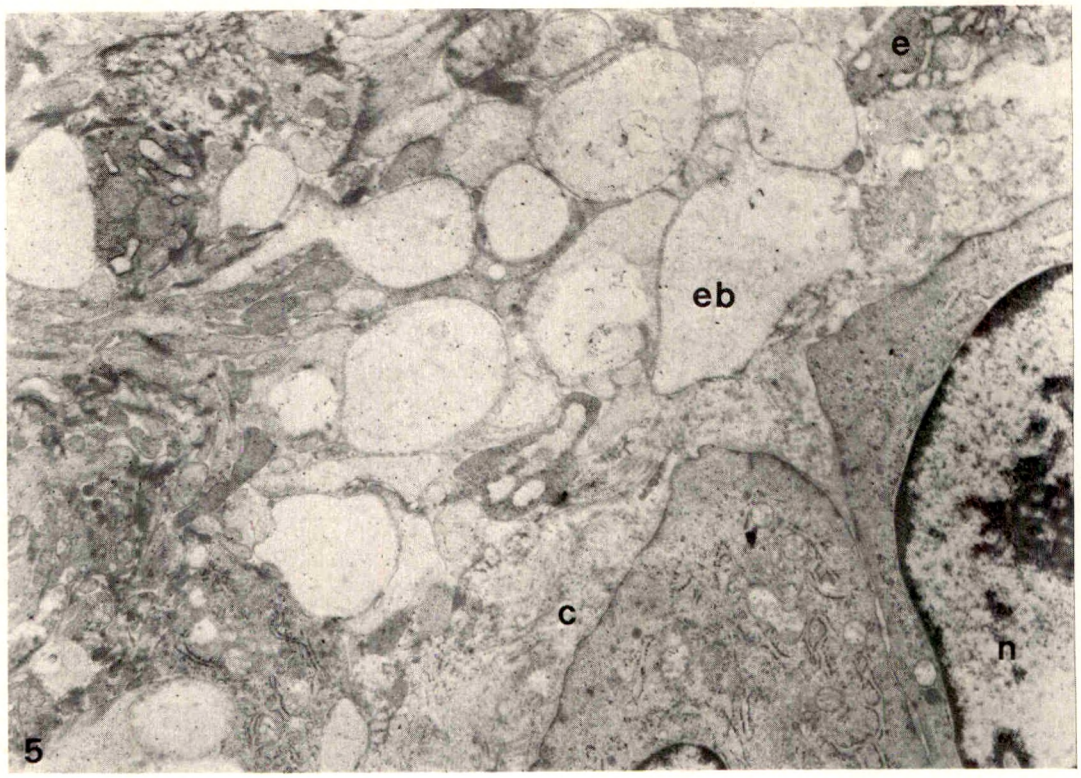


Fig. 5. Electron micrograph of area in *box* in Fig. 4 showing disrupted endothelium *(e)*. Empty balloons *(eb)* are attached to subendothelial collagen *(c)*. *n*, Nucleus of subendothelial cell. (Original magnification ×11,880.)

widely used inhibitor, mefenamic acid, on menstrual blood loss and on the morphologic features of early menstrual hemostasis in menorrhagic women. Mefenamic acid and placebo were used in a double blind study. Mefenamic acid decreased menstrual blood loss by 40%, which is in accordance with previous reports.[1, 2]

The morphologic features of early menstrual hemostasis of both groups were strikingly different. In the mefenamic acid group, fewer huge hemostatic plugs were observed and their extravascular extensions were smaller. Hemostatic plugs in the mefenamic acid group were further transformed than those in the placebo group. Three other significant differences of interest were found in the morphometric studies. First, the number of vessels without a hemostatic plug was decreased in the mefenamic acid group. Second, the vessel area occluded by a plug in venules was increased in the mefenamic acid group. Third, the degree of hemostatic plug transformation correlated inversely with the decrease in menstrual blood loss in the mefenamic acid group.

The differences described here suggest that mefenamic acid may act through vasoconstriction and improvement of platelet aggregation and degranulation. These changes obviously decrease the amount of menstrual blood loss.

The observed changes may have been mediated by inhibition of prostaglandin I_2 and E_2 synthesis. An effect of mefenamic acid or other nonsteroidal antiinflammatory drugs on fibrinolysis has never been described. Recanalization of plugs, reflecting increased fibrinolysis, was seen as frequently in the venules of the placebo group as in the mefenamic acid group. In arterioles, recanalized plugs were seen significantly more often in the mefenamic acid group, but this is of little value because of their small number.

The effect of mefenamic acid on menstrual hemostasis in essential menorrhagia is strikingly different from the effect of acetylsalicylic acid in human skin wounds.[17] Acetylsalicylic acid caused impairment of platelet aggregation and degranulation. The discrepancy may be the result of a different prostaglandin metabolism in the human skin compared with menstrual endometrium of menorrhagic women, or by a different mode of action between acetylsalicylic acid and mefenamic acid. The fact that acetylsalicylic acid does not decrease menstrual blood loss[18] is an argument for the latter hypothesis.

How precisely mefenamic acid causes vasoconstriction and further transformation of hemostatic plugs remains to be answered. Granström et al.[19] have suggested that under the influence of prostaglandin synthesis inhibitors on cyclooxygenase, arachidonic acid is predominantly metabolized toward leukotrienes via the lipoxygenase pathway. Leukotrienes are strong vaso-

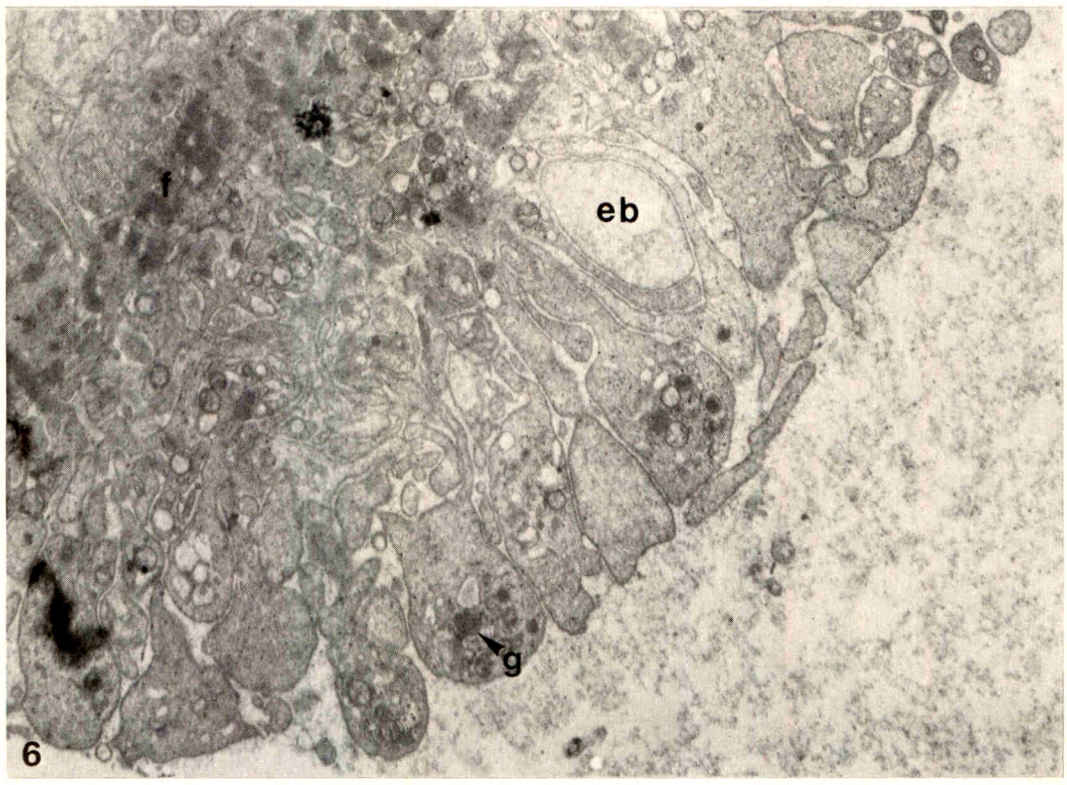


Fig. 6. Electron micrograph of periphery of hemostatic plug from patient receiving mefenamic acid showing firm interdigitation of degranulated platelets, empty balloon *(eb)*, and centrally located fibrin *(f)*. *g*, Granule. (Original magnification ×15,100.)

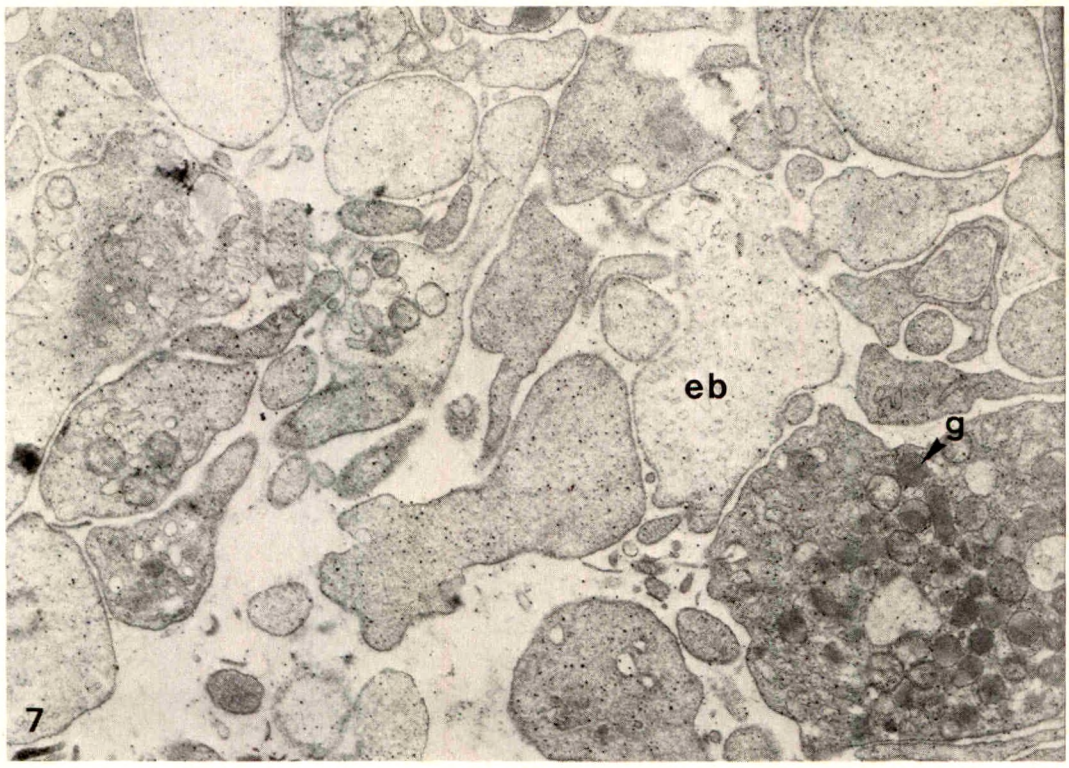


Fig. 7. Electron micrograph of hemostatic plug from patient receiving placebo showing poor interdigitating platelets. Some platelets still contain granules *(g)*. No fibrin fibers are seen. *eb*, Empty balloon. (Original magnification ×17,700.)

constrictors and would reduce menstrual blood loss. Our findings are in agreement with such a hypothesis. Additionally, we also found an improvement of the transformation of the hemostatic plug in menstrual endometrium of women receiving mefenamic acid. How this is related to prostaglandin metabolism in endothelial cells and platelets deserves further study.

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Elevated plasma Met-enkephalin levels in the human newborn are a poor indicator of perinatal stress

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OBJECTIVE: This study was designed to investigate whether plasma Met-enkephalin peptides could serve as markers of physiologic stress in the neonate.

STUDY DESIGN: Infants (n = 115) between 1.2 and 4.7 kg and 28 and 42 weeks of gestation were studied at birth. Seventy-four infants were delivered by the vaginal route, 31 by cesarean section after labor, and 10 by cesarean section before labor. Correlations were sought between plasma enkephalin peptides and epinephrine, norepinephrine, and arterial blood gases with linear regression analysis. Various clinical data were also analyzed.

RESULTS: Plasma Met-enkephalin levels were significantly greater in infants exposed to labor (440 ± 36 vs 260 ± 30 pg/ml, $p < 0.05$). The large-molecular-weight forms of enkephalin peptides were also greater in these infants (approximately 50 ± 4 vs 23 ± 2 ng/ml). There was no correlation between plasma enkephalin peptides and catecholamines, arterial pH, or Apgar scores. There was a significant but weak correlation between plasma Met-enkephalin levels and birth weight ($r = 0.34$, $p = 0.03$) and Pao_2 ($r = -0.28$, $p < 0.05$).

CONCLUSION: The lack of correlation between Met-enkephalin plasma levels and umbilical plasma catecholamine concentrations, acid-base status, or Apgar scores suggests that circulating Met-enkephalin is a poor indicator of stress in the newborn. (Am J Obstet Gynecol 1992;166:1429-35.)

Key words: Met-enkephalin, catecholamines, umbilical cord blood

The enkephalin peptides are a family of related peptides that include Met-enkephalin, Leu-enkephalin, larger-molecular-weight carboxy-extended forms of Met-enkephalin, and the prohormone proenkephalin A.[1] Investigators have shown cosecretion of discrete forms of the enkephalin peptides with catecholamines into the circulation in response to diverse stimuli.[2-4] We have previously shown plasma Met-enkephalin levels are elevated in a group of healthy newborn infants at the time of birth when compared with adult volunteers.[5]

Numerous investigators have reported elevated levels of plasma catecholamines at birth.[6, 7] In addition, we demonstrated by multiple regression analysis that among a variety of clinical variables umbilical arterial plasma catecholamines correlated best with the degree of acidemia in umbilical cord blood at delivery.[7] The current study was designed to investigate whether measurement of plasma enkephalin peptides could be used as a marker of physiologic stress in the neonate. We sought to find correlations between enkephalin peptides with characteristics of labor, route of delivery, arterial blood gases, and plasma catecholamines. In addition, we evaluated correlations between circulating plasma enkephalin peptides and clinical characteristics of newborn infants.

Material and methods

Study protocol. Institutional review board approval was obtained to collect umbilical cord blood from infants born at Harbor–University of California, Los Angeles Medical Center. Blood was drawn from the placenta immediately after cord clamping for measurement of plasma Met-enkephalin, larger-molecular-weight Met-enkephalin-containing peptides, plasma catecholamines, and arterial blood gas analysis. Clinical data were obtained from the mothers' and infants' medical records and included presence and duration of labor, Apgar scores, fetal heart tracing, birth weight, gestational age, and route of delivery.

Laboratory analysis. Blood for measurement of Met-enkephalin was immediately placed into chilled test tubes containing sodium citrate (0.5 mmol/L, final concentration), aprotinin (500 U/ml), and ethylenedi-

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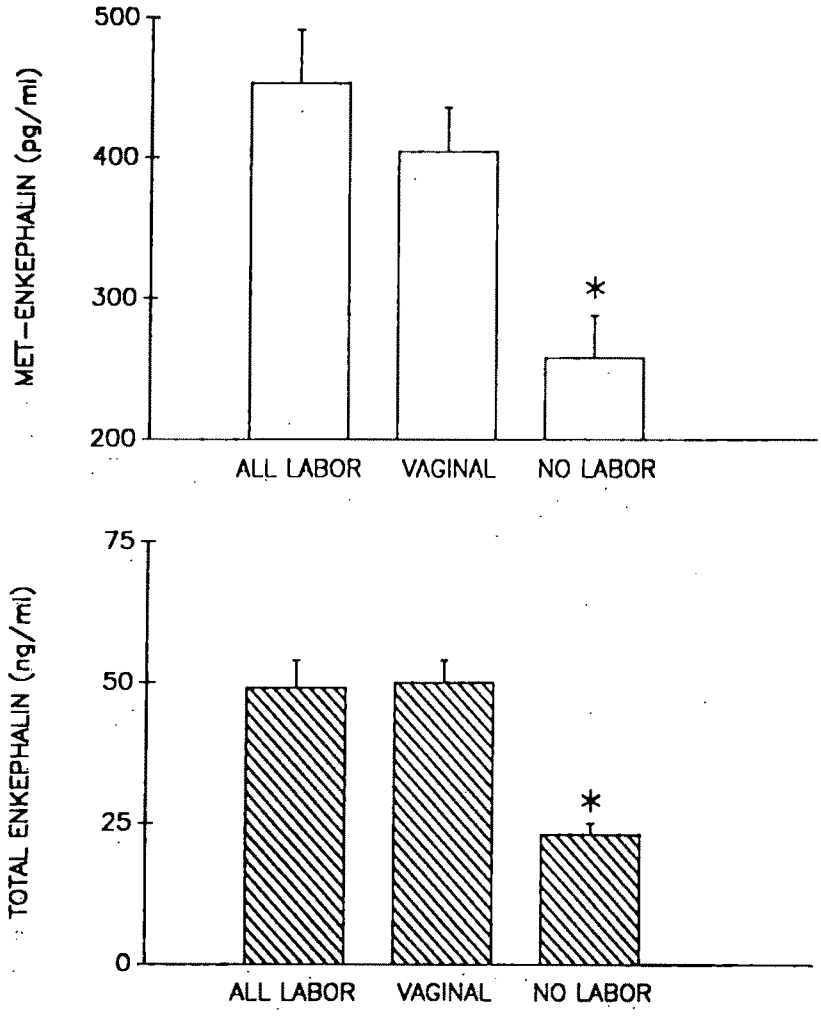


Fig. 1. Umbilical cord plasma Met-enkephalin immunoreactivity *(upper panel)* and high-molecular-weight enkephalin peptide *(TOTAL ENKEPHALIN) (lower panel)*. Values are mean ± SEM. *Asterisk*, $p < 0.05$ (Mann-Whitney nonparametric test).

Table I. Characteristics of 115 patients studied

	Group 1 (n = 105)	*Group 2 (n = 74)*	*Group 3 (n = 10)*
Weight (kg)	3.3 ± 0.1 (1.2-4.7)	3.3 ± 0.1 (1.4-4.7)	2.8 ± 0.3 (1.4-4)
Gestational age (wk)	38.8 ± 0.3 (28-44)	39.3 ± 0.3 (29-43)	37 ± 2 (30-42)
Pao_2 (mm Hg)	18.9 ± 1 (8-52)	19 ± 1 (9-36)	19.1 ± 2 (11-32)
pH	7.23 ± 0.01 (7.02-7.4)	7.23 ± 0.01 (7.0-7.4)	7.2 ± 0.6 (6.8-7.3)
Apgar score (range)			
1 min	1-9	1-9	1-9
5 min	5-10	5-10	1-10

Group 1, Infants exposed to labor; *group 2*, vaginal deliveries; *group 3*, cesarean section with no labor. Data are mean ± SEM with range shown in parentheses.

aminetetraacetic acid (3 mmol/L). Arterial blood for measurement of catecholamines was immediately placed into chilled tubes containing reduced glutathione (3 mmol/L) and ethylene glycol-bis(β-aminoethyl ether)N,N,N′,N′-tetraacetic acid (4 mmol/L). All samples were centrifuged, and the plasma was separated and frozen at −70° C for analysis.

Plasma for Met-enkephalin analysis was first extracted with polystyrene columns and then measured by radioimmunoassay as previously described.[8] Because the antiserum used cross reacts 2.8% with Leu-enkephalin, the data represent Met-enkephalin immunoreactivity. Plasma measurement of the Met-enkephalin immunoreactivity released from the high-molec-

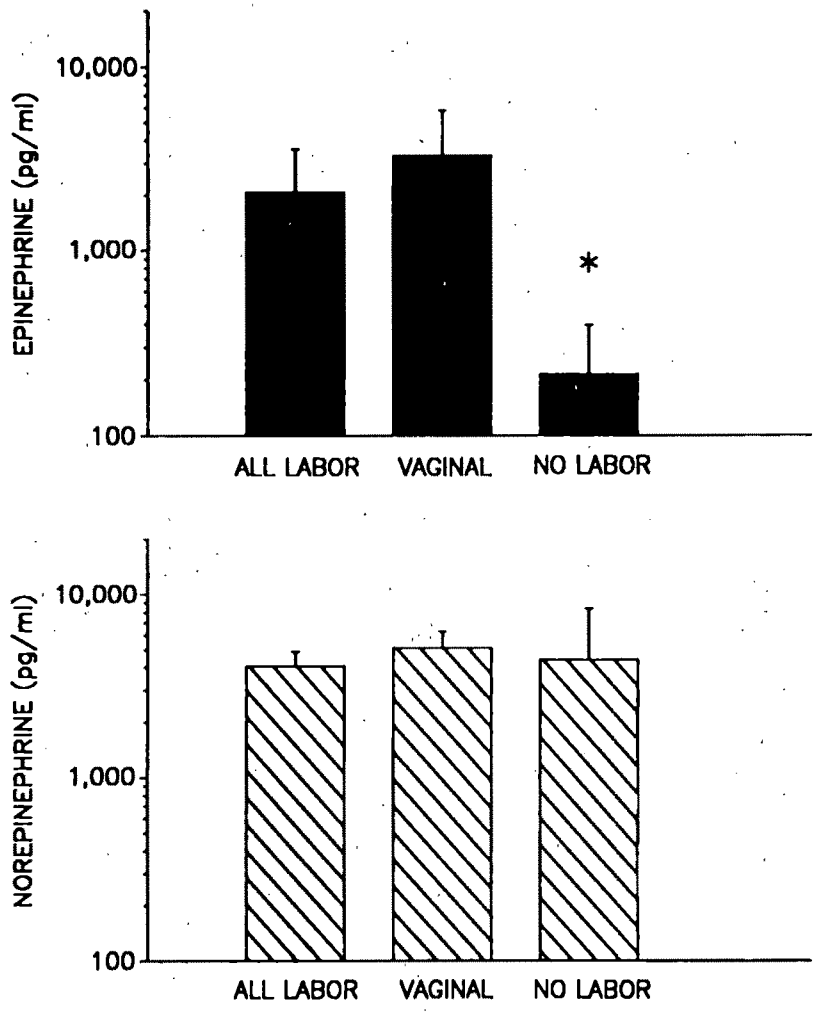


Fig. 2. Umbilical cord plasma epinephrine *(upper panel)* and norepinephrine *(lower panel)*. *Asterisk*, $p < 0.05$ (Mann-Whitney).

ular-weight enkephalin peptides first underwent proteolytic digestion with trypsin and carboxypeptidase B, as previously described.[2,8] The plasma was extracted and measured by radioimmunoassay as described above. The assay is sensitive to 2 pg. The interassay and intraassay variabilities were 13% and 9%, respectively. Recovery of Met-enkephalin with this assay was determined to be >90% after 1000, 500, or 200 pg/ml additions to unextracted plasma.

Plasma catecholamines were measured by a radioenzymatic assay sensitive to 1 to 2 pg of norepinephrine and epinephrine, as previously described.[9]

Data analysis. The data are presented as mean and SEM. The catecholamine data were log transformed for statistical analysis. The Mann-Whitney nonparametric test was used for comparisons between the different groups studied. Linear regression and stepwise multiple regression analysis was used to compare the enkephalin levels with clinical parameters and hormonal measurements. A value of $p < 0.05$ was considered statistically significant.

Results

One hundred fifteen infants were studied. Seventy-four were born by vaginal route and 31 by cesarean section after a trial of labor and 10 without any exposure to labor. In Table I the infants are divided into infants exposed to labor (group 1), infants delivered vaginally (group 2), and infants delivered by cesarean section without labor (group 3). The birth weights of the infants ranged from 1.2 to 4.7 kg, and the gestational ages ranged from 28 to 42 weeks. Table I also shows additional information obtained from the maternal records, including birth weight, gestational age, arterial pH and PaO_2, and Apgar score. As can be seen from the range of values, this was a diverse group of infants, some of whom were quite ill at the time of delivery.

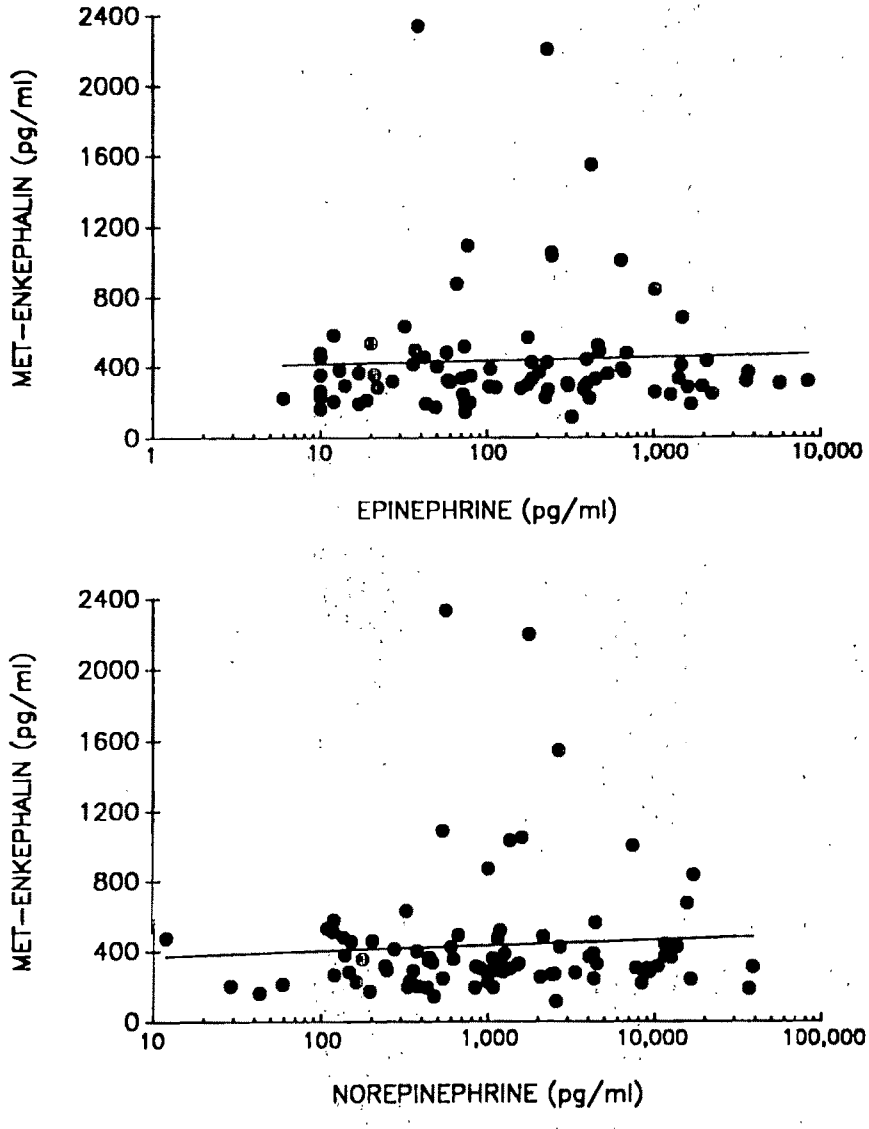


Fig. 3. Linear regressions between Met-enkephalin immunoreactivity and plasma epinephrine *(upper panel)* and norepinephrine *(lower panel)* in 87 paired samples.

The plasma enkephalin peptide levels are shown in Fig. 1. Plasma Met-enkephalin immunoreactivity levels were significantly greater in those infants undergoing labor before delivery, with levels of 450 ± 40 pg/ml for all labor and 400 ± 30 pg/ml for vaginal delivery versus 260 ± 30 pg/ml for patients delivered with no labor. Similarly, the high-molecular-weight enkephalin peptide levels were higher in infants born after labor when compared with infants born with no exposure to labor, with levels of approximately 50 ± 4 versus 23 ± 2 ng/ml.

As can be seen in Fig. 2, plasma epinephrine levels were similarly elevated in infants born after labor, with levels of 3300 ± 2400 and 4000 ± 3000 pg/ml versus levels of 300 ± 250 pg/ml in those infants born by cesarean section with no labor. There were no differences in plasma norepinephrine levels among the three groups of infants.

With linear regression in 87 paired samples we could find no correlation between high-molecular-weight enkephalin peptide and plasma epinephrine ($r = -0.102$, $p = 0.35$) or norepinephrine ($r = -0.06$, $p = 0.6$). These data are shown graphically in Fig. 3. Although not shown, there was similarly no correlation between high-molecular-weight enkephalin peptide versus epinephrine or norepinephrine. Using stepwise multiple regression analysis we found weak but significant correlations between Met-enkephalin immunoreactivity and birth weight and Pao_2 ($p < 0.05$). A second-order equation best fit the data and is shown in Fig. 4.

Comment

This study was undertaken to determine whether measurement of plasma Met-enkephalin or enkephalin-related peptides could be used as markers of phys-

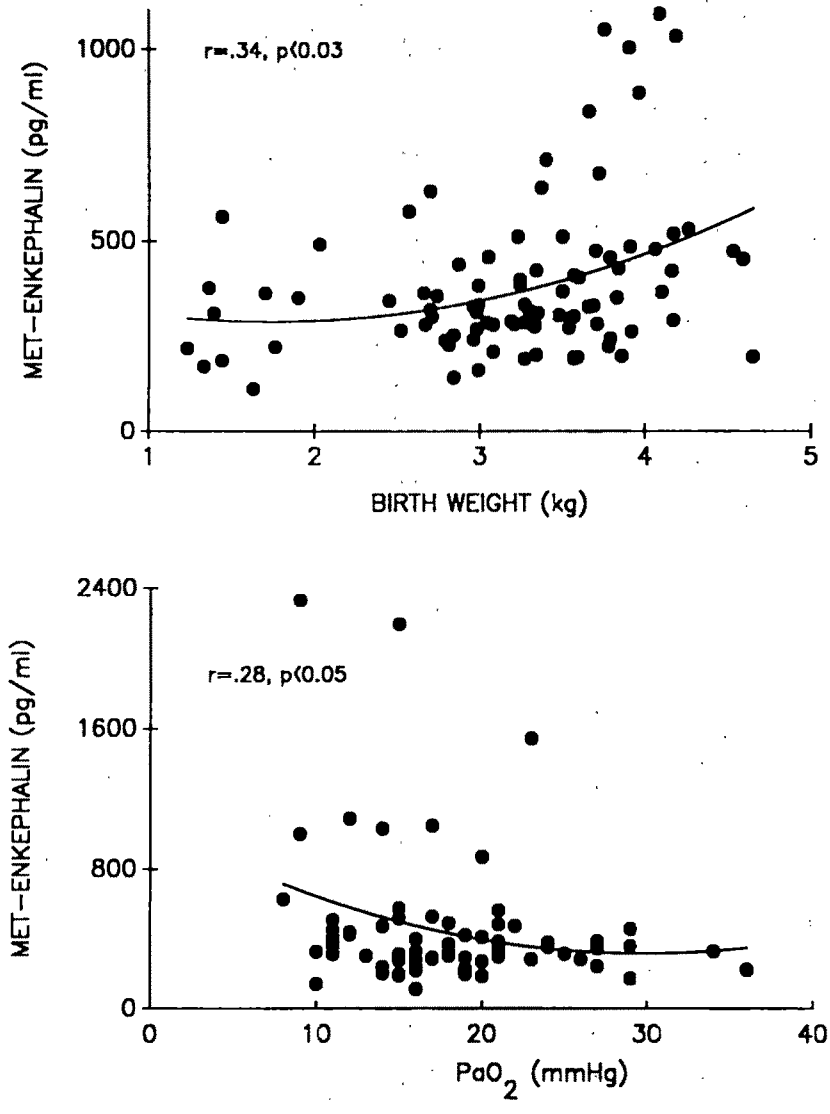


Fig. 4. Linear regressions between Met-enkephalin immunoreactivity and birth weight *(upper panel)* and PaO_2 *(lower panel)* in 87 paired samples.

iologic stress in the newborn at birth. We asked whether circulating enkephalin peptides would correlate with established markers of perinatal stress, such as arterial blood gases and plasma catecholamines. In addition, we sought to find significant correlations between circulating enkephalin peptides and clinical characteristics of newborn infants, such as gestational age, birth weight, Apgar scores, and mode of delivery. We had previously reported plasma high-molecular-weight enkephalin peptide levels to be significantly greater in a smaller group of healthy newborn infants' umbilical cord blood when compared with that of nonpregnant adult volunteers, whose plasma levels were approximately 25 ± 2 pg/ml. The plasma high-molecular-weight enkephalin peptide levels were not elevated in these newborn infants when compared with adult levels.[5]

In the current study we found that plasma Met-enkephalin immunoreactivity and high-molecular-weight enkephalin peptide levels were significantly greater in infants born after a trial of labor. We similarly found plasma epinephrine levels to be greater in these same infants. In addition, we found significant but weak correlations between plasma Met-enkephalin immunoreactivity versus birth weight and arterial PaO_2. We found no correlation between plasma levels of Met-enkephalin immunoreactivity or high-molecular-weight enkephalin peptide versus plasma epinephrine or norepinephrine, systemic pH, or Apgar scores.

The opiate peptides (which include enkephalins, endorphins, and dynorphins) have been implicated in the response to diverse forms of stress, including hypoxia, hypoglycemia, hemorrhage, and septic shock.[8, 10, 11] The opiate peptides and their receptors have been identified in central nervous system areas important for autonomic, cardiovascular, and respiratory control.[10] Recent

investigations have suggested the opiate peptides may play a role in the response to asphyxia and hypoxia in the fetal and neonatal period.[15] β-Endorphin is elevated during pregnancy, in response to labor and delivery.[16] An inverse correlation between β-endorphin and umbilical cord PaO_2 and pH have been previously shown in both sheep and human studies.[15, 16] β-Endorphins have also been shown to be elevated in term and premature infants born with perinatal depression.[17] By contrast, the role of the enkephalin peptides during delivery and perinatal stress has not been fully examined.

The physiologic role of opiate peptides has been the subject of many investigations. In vitro studies have shown that opiate peptides inhibit norepinephrine release in sympathetic ganglia through presynaptic inhibition of cholinergic transmission.[18] Opiate peptides have also been shown to inhibit adrenal catecholamine release in response to cholinergic stimulation.[19] In vivo studies with the opiate receptor antagonist, naloxone, have shown increases in circulating catecholamines and improvements in blood pressure and circulatory function in different animal models of shock.[10, 11, 20] We have shown that opiate peptide receptor blockade with naloxone augments epinephrine levels in fetal sheep exposed to hypoxia[21] and augments catecholamine levels and cardiovascular responses in newborn sheep at birth.[22]

The enkephalins are localized in brain stem nuclei involved in autonomic regulation, in sympathetic neurons and sympathetic ganglia, and in chromaffin cells of the adrenal medulla.[23, 24] In the adrenal medulla the enkephalin peptides are costored with catecholamines as Met-enkephalin and Leu-enkephalin and as larger-molecular-weight peptides, including the prohormone proenkephalin A.[1] All forms of enkephalin peptides have been shown to be coreleased with catecholamines from the adrenal gland during physiologic stimuli.[2] In this current study we found concomitant elevations of plasma Met-enkephalin peptides and catecholamines at birth. In addition, the highest levels of enkephalin peptides and epinephrine are found in those infants exposed to labor. These data do not negate or prove that cosecretion of these peptides occurs at birth. Differences in the secretion rates or plasma clearance rates of these peptides in the newborn may account for the lack of correlation seen in the circulating levels. These unresolved questions would represent exciting directions for future studies.

The increase in plasma Met-enkephalin seen in the perinatal period is consistent with the postulated role the opiate peptides have in the ventilatory and cardiovascular responses in the perinatal period.[12-14, 25, 26] The lack of correlation between Met-enkephalin plasma levels with catecholamines or systemic pH and the poor correlation with arterial PaO_2 suggests circulating Met-enkephalin is a poor marker of stress in the perinatal period. Furthermore, because proenkephalin A is only expressed in neural tissue (sympathetic neurons, ganglia, adrenal medulla, and the central nervous system), we speculate that the correlation seen in this study with birth weight may represent a neuromaturational phenomenon.

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Lack of reactivity of uterine arteries from patients with obstetric hemorrhage

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Obstetric hemorrhage may occur throughout pregnancy and the puerperium. The purpose of this study was to investigate the reactivity of isolated, suffused uterine arteries from obstetric patients with uncontrollable uterine bleeding and to compare those blood vessels with uterine arteries from patients undergoing cesarean hysterectomy for other medical reasons (control patients). The uterine arteries from the control patients (n = 9) responded with maximal or near-maximal constriction to norepinephrine (30 μmol/L, 3.6 ± 1 gm), potassium chloride (75 mmol/L, 10.2 ± 3 gm), prostaglandin $F_{2\alpha}$ (30 μmol/L, 1.8 ± 1 gm), and arginine vasopressin (1 μmol/L, 18.8 ± 2.6 gm). In uterine arteries from five patients with uncontrollable bleeding, the constrictor responses to the same drugs were markedly depressed: norepinephrine (30 μmol/L, 0.5 ± 0.2 gm), potassium chloride (75 mmol/L, 1.9 ± 0.8 gm); prostaglandin $F_{2\alpha}$ (30 μmol/L, 0 gm), and arginine vasopressin (1 μmol/L, 0.2 ± 0.05 gm). Uterine arteries from two patients exhibited no constrictor responses to norepinephrine (30 μmol/L), potassium chloride (75 mmol/L), prostaglandin $F_{2\alpha}$ (30 μmol/L), or arginine vasopressin (1 μmol/L). The impaired responses to the vasoconstrictor drugs were not reversed by indomethacin (1 μmol/L), which is an inhibitor of prostaglandin synthetase; methylene blue (10 μmol/L), which is a blocker of endothelium-derived relaxing factor activation of guanylate cyclase; or propranolol (1 μmol/L), a β-adrenergic receptor antagonist. The levels of adenosine 3′:5′-cyclic monophosphate were not elevated in the uterine arteries from the patients with obstetric hemorrhage. The impaired reactivity to the multiple vasoconstrictors implies that a mechanism involved in constriction common to all of the constrictors is depressed or blocked. Furthermore, the depression or lack of reactivity of these isolated uterine arteries is not mediated by vasodilatory prostaglandins, endothelium-derived relaxing factor, β-adrenergic receptors, or elevated levels of adenosine 3′:5′-cyclic monophosphate. The results suggest that obstetric hemorrhage involves, in part, a lack of constrictor reactivity of the uterine vasculature. (Am J Obstet Gynecol 1992;166:1436-43.)

Key words: Vascular smooth muscle, norepinephrine-induced constriction, potassium chloride–induced constriction, obstetric hemorrhage, pregnancy

Obstetric hemorrhage may occur throughout pregnancy and the puerperium. Postpartum hemostasis is generally thought to result from uterine muscle contraction and retraction and from thrombosis and fibrosis. Causes of the loss of this hemostasis, which results in uncontrollable uterine bleeding, include uterine atony, trauma to the genital tract and adjacent structures during labor and delivery, retained products of conception, coagulation defects, and abnormal placentation.[1] The management in cases of hemorrhage includes obstetric and pharmacologic maneuvers such as uterine massage, exploration and curettage of the uterus, surgical repair of traumatized tissue, and administration of ergots, oxytocin, prostaglandins, and other pressor drugs, as well as the administration of electrolytes, blood, and blood components.[1] If these measures are unsuccessful, emergency hysterectomy may be required.

Although a number of factors are associated with obstetric hemorrhage, virtually nothing is known about the reactivity of the uterine vasculature during uncontrollable uterine bleeding of pregnant patients. Uncomplicated (normal) pregnancy has been shown to have little or no effect on the responses of the human uterine artery to various constrictor agents.[2, 3] The possibility exists that the uterine vasculature of pregnant patients with uncontrollable uterine bleeding is depressed and thus does not constrict sufficiently for hemostasis. The purpose of this study was to investigate the reactivity of uterine arteries from patients with obstetric hemorrhage who underwent cesarean hysterectomy and to compare those blood vessels with uterine arteries from patients who underwent cesarean hysterectomy for other medical reasons (control patients). The use of uterine arteries obtained exclusively from pregnant patients avoided confounding hemodynamic, humoral,

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and neurogenic influences of the nonpregnant condition.

A number of mechanisms responsible for the uterine vasodilatation that occurs during normal pregnancy have been investigated. For example, studies have shown that pregnancy increases the release from the endothelium of vasodilator substances such as endothelium-derived relaxing factor in the uterine artery from humans[4] and guinea pigs[5] or prostacyclin in the uterine artery from sheep.[6] Other studies suggest an alteration in calcium channels or intracellular calcium stores in the isolated uterine artery from the pregnant human.[2] Drug-induced increases in levels of adenosine 3′:5′-cyclic monophosphate (cAMP) are associated with relaxation of smooth muscle, and elevated basal levels of cAMP have been reported in uterine arteries from pregnant ewes.[7] Any of these mechanisms involved in decreasing uterine vascular tone during normal pregnancy may play an even greater role in uterine arteries from patients with obstetric hemorrhage.

Material and methods

Specimens of the ascending branch of the uterine artery were obtained from patients undergoing cesarean hysterectomy for various medical reasons. Use of the uterine arteries from patients undergoing hysterectomy was approved by the Institutional Review Board for the University of Texas Medical Branch, Galveston, Tex. Patients varied in age from 23 to 37 years. All gestations were term. The patients received antacid (Bicitra, Willen Drug Co.) for aspiration prophylaxis. Anesthesia was induced with thiopental and maintained with nitrous oxide–oxygen and either halothane, enflurane, or isoflurane.

Sections of the uterus that contained the uterine arteries were placed in gassed (95% oxygen and 5% carbon dioxide) Krebs-bicarbonate solution immediately after hysterectomy and before being taken to the laboratory. The preparation of isolated arterial rings and the suffusion techniques used have been described in detail previously.[8,9] In brief, rings 2 mm in length were prepared from the uterine artery and mounted between two tungsten wires in a 5 ml volume suffusion chamber. The diameter, measured by a dissecting microscope, of the unstimulated uterine artery taken from patients with uncomplicated pregnancy is not significantly different when compared with the uterine artery from multiparous, nonpregnant patients. Changes in isometric tension were measured by means of a force-displacement transducer (Statham) connected to the upper mounting wire and recorded on a Gould recorder (model 2400, Brush).

The mounted arteries were suffused continuously with gassed (95% oxygen and 5% carbon dioxide) Krebs-bicarbonate solution at a flow rate of 4 ml/min delivered by a peristaltic pump (Gilson Instruments). The Krebs-bicarbonate solution had the following composition: sodium chloride, 119 mmol/L; sodium bicarbonate, 25 mmol/L; potassium chloride, 3.6 mmol/L; magnesium sulfate, 1.2 mmol/L; potassium phosphate monobasic, 1.2 mmol/L; calcium chloride, 2.5 mmol/L; glucose, 11 mmol/L; ascorbic acid, 0.005 mmol/L; and disodium ethylenediaminetetraacetate, 0.03 mmol/L. The bath solution was maintained at 37° C and had a pH of 7.4. The drugs were adminstered in the inflowing suffusion fluid.

The preparation was allowed a stabilization period of 1 to 2 hours before the experiment began. During that time, basal tension was adjusted repeatedly until the resting tension became stable at 1 gm, which we have found to be the optimal resting tension for the human uterine arteries. Uterine arteries from patients having cesarean hysterectomy for medical reasons other than obstetric hemorrhage (control patients, type I) had reproducible constrictor responses to norepinephrine for several hours after the stabilization period.

The drugs studied, including norepinephrine, serotonin, arginine vasopressin, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and potassium chloride have multiple mechanisms of constriction, such as activation of receptor-operated and voltage-dependent calcium channels, as well as release of intracellular calcium. The concentrations of the drugs used in the studies were found to elicit 60% to 100% of the maximum constriction produced by each drug in uterine arteries from normal pregnant and nonpregnant patients. If little or no constriction was elicited by the vasoconstrictor drug, the drug concentration was increased. Each concentration of the constrictor drug was administered for at least 15 minutes, a time found to produce a stable constriction in uterine arteries from normal pregnant patients.[2] Each drug was washed out for 30 minutes before the subsequent administration of another drug.

The mechanisms for the impaired constrictor responses were investigated with the following procedure. Norepinephrine (30 μmol/L) was administered for 15 minutes. After the norepinephrine-induced constriction returned to baseline or after 15 minutes, the arterial ring was suffused for 30 minutes with a given concentration of indomethacin, to inhibit prostaglandin synthetase, with methylene blue, to prevent the effect of endothelium-derived relaxing factor on soluble guanylate cyclase, or with propranolol, to antagonize β-adrenergic receptors. Norepinephrine, in the presence of the antagonist or blocker, was again administered for 15 minutes.

To determine whether the impaired constrictor responses were due to elevated basal levels of cAMP that are associated with smooth muscle relaxation, basal

Table I. Categories of patients on basis of reactivity of isolated uterine arteries

Patient No.	*Lowest MAP (mm Hg)*	*Diagnosis*	*Estimated blood loss (ml)*	*Coagulopathy*	*Drugs*	*Associated clinical phenomena*
Type I (normal reactivity)						
03	84	Placenta accreta	–	–	—	Repeat cesarean section
04	83	Fibroids	–	–	—	—
65	80	Cervical carcinoma, marginal placenta previa	–	–	Levothyroxine sodium (Synthroid)	Amniocentesis
74	85	Cervical cancer	–	–	—	Amniocentesis
72	97	Severe dysplasia	–	–	—	Repeat cesarean section, amniocentesis
71	96	Carcinoma	–	–	—	—
77	93	Placenta accreta	–	–	—	Chronic hypertension, toxemia with previous pregnancy, repeat cesarean section, amniocentesis
22	85	Permanent sterilization	–	–	—	Repeat cesarean section
83	84	Cervical carcinoma	–	–	Terbutaline, folic acid	Amniocentesis, anemia
Type II (weak reactivity)						
48	73	Repeat cesarean section	900	–	Oxytocin (Pitocin)	Amniocentesis, anemia
64	53	Uterine atony, possible amniotic fluid embolism	10,000	+ (DIC)	Methylergonovine maleate (Methergine), $PGF_{2\alpha}$, blood products, furosemide (Lasix), gentamicin, dopamine, Pitocin	—

All patients were multiparous and had no evidence of hypoxia or sepsis. Coagulopathy was determined by prothrombin time, thromboplastin time, platelet levels, levels of fibrinogen, and fibrin degradation products. Drugs included medications other than those normally used. *MAP*, Lowest mean arterial pressure; *DIC*, disseminated intravascular coagulation.

levels (but not stimulated levels) of cAMP were determined by radioimmunoassay.[10, 11] Stimulated levels of cAMP were not determined because the depessed contractility precluded a correlation between vasorelaxation and stimulated levels of cAMP. Portions of arteries were blotted, weighed, and frozen in liquid nitrogen. Frozen tissue was homogenized in 2 ml of 10% trichloroacetic acid (cold) with a Polytron tissue disruptor at a setting of 6 for 30 seconds. The supernatant was lyophilized and reconstituted in 1 ml of 50 mmol/L sodium acetate, pH 4.75. Aliquots were acetylated and assayed for cAMP by radiommunoassay.

Because endogenous vasodilators and vasoconstrictors may be released from the endothelium, the presence of endothelium was determined by a silver stain technique.[12] Arterial rings proximal and distal to the rings used for the pharmacologic studies were cut open, laid adventitia-side-down, and stained. Other rings were stored in 10% formalin for subsequent paraffin embedding and staining with hematoxylin and eosin.

Norepinephrine-induced constriction was often associated with phasic constrictions (drug-induced oscillatory activity). When norepinephrine-induced oscillatory activity occurred, the lowest tension that developed during the rhythmic activity was taken as the response (i.e., the amplitude of the rhythmic activities was not included in the measurements). Data are presented as mean values ± SE.

All stock solutions and serial dilutions made in Krebs-bicarbonate solution were prepared shortly before the start of each of the experiments. The following drugs were used and were obtained from Sigma Chemical Company, St. Louis: (−)-arterenol bitartrate, indomethacin, and methylene blue, $PGF_{2\alpha}$, [Arg^8]-vasopressin acetate, DL-propranolol hydrochloride, histamine diphosphate, and 5-hydroxytryptamine creatinine sulfate.

Results

The patients were divided into three categories. The control patients were considered type I. The hemorrhaging patients were divided into types II and III on

Table I—Cont'd

06	57	Retained products of conception	4000	Thrombocytopenia	$PGF_{2\alpha}$, blood products, ampicillin, gentamicin	History of intravenous drug abuse and hepatitis B, chronic inflammation of endometrium and myometrium, lymphocyte infiltration of myometrium
75	66	Placenta accreta, secondary vaginal and uterine laceration	5000	+ (DIC)	Methergine, $PGF_{2\alpha}$, blood products, gentamicin, (Cleocin), magnesium sulfate	Delivery at home, breech birth
82	77	Uterine atony	3200	+ (DIC)	Methergine, $PGE_{2\alpha}$, ephedrine, Pitressin, blood products, albumin, magnesium sulfate, hydralazine	Repeat cesarean section, chronic anemia, chronic cervicitis
Type III (no reactivity)						
01	88	Abnormal placental implantation	2000	–	Pitocin, $PGF_{2\alpha}$, prostaglandin E_2	—
26	76	Uterine atony	1500	–	Methergine, $PGF_{2\alpha}$, prostaglandin E_2, Pitocin	Amniocentesis, repeat cesarean section, anemia

the basis of reactivity of the isolated uterine artery. Types II and III were not associated with any particular diagnosis, parity, presence of coagulopathies, or depressed mean arterial blood pressure (Table I).

The uterine arteries from the control patients (type I, $n = 9$) responded with a maximum or near-maximum constriction to norepinephrine (30 μmol/L, 3.6 ± 1 gm), potassium chloride (75 mmol/L, 10.2 ± 3 gm), and arginine vasopressin (1 μmol/L, 18.8 ± 2.6 gm). In uterine arteries from five patients with uncontrollable bleeding (type II), the constrictor responses to the same drugs were markedly depressed: norepinephrine (30 μmol/L, 0.5 ± 0.2 gm), potassium chloride (75 mmol/L, 1.9 ± 0.8 gm), $PGF_{2\alpha}$ (30 μmol/L, 0 gm) and arginine vasopressin (1 μmol/L, 0.2 ± 0.05 gm). Uterine arteries from two patients (type III) exhibited no constrictor responses to norepinephrine (30 μmol/L), potassium chloride (75 mmol/L), $PGF_{2\alpha}$ (30 μmol/L), or arginine vasopressin (1 μmol/L). Records of experiments that used uterine arteries from the three categories of patients are shown in Fig. 1. The results from the experiments are summarized in Fig. 2. When the concentrations of the constrictor drugs were increased, little or no further increases in constriction were observed. In preliminary studies that used uterine arteries from patients with uncontrollable uterine bleeding various concentrations of the drugs were used but no responses were elicited except at higher concentrations. The depressed responses to the various vasoconstrictors persisted, in spite of continual suffusion and repeated administration of the drugs for 8 to 12 hours.

Other constrictor agents (serotonin, 30 μmol/L; calcium chloride, 4 mmol/L; histamine, 30 μmol/L) were used in a few of the uterine arteries from patients with obstetric hemorrhage (types II and III). None of these agents induced constriction. The impaired response to norepinephrine was not reversed by indomethacin (1 μmol/L), which is an inhibitor of prostaglandin synthetase; by methylene blue (10 μmol/L), which is a blocker of endothelium-derived relaxing factor activation of guanylate cyclase; or by propranolol (1 μmol/L), an antagonist of β-adrenergic receptors (Fig. 3).

The basal concentrations of cAMP were 152.7 ± 1.4 pmol/gm wet weight in uterine arteries ($n = 3$) from patients with obstetric hemorrhage and 170.3 ± 4.5 pmol/gm wet weight in uterine arteries ($n = 3$) from control patients.

The intimal surface of the arterial rings was examined microscopically *en face* after application of a silver stain to ascertain the presence of endothelial cells. Almost all the intimal surface of the rings was covered by endothelial cells as demonstrated by "black" cell borders. In the tissues that were embedded, stained, and examined at a magnification of ×40, there did not

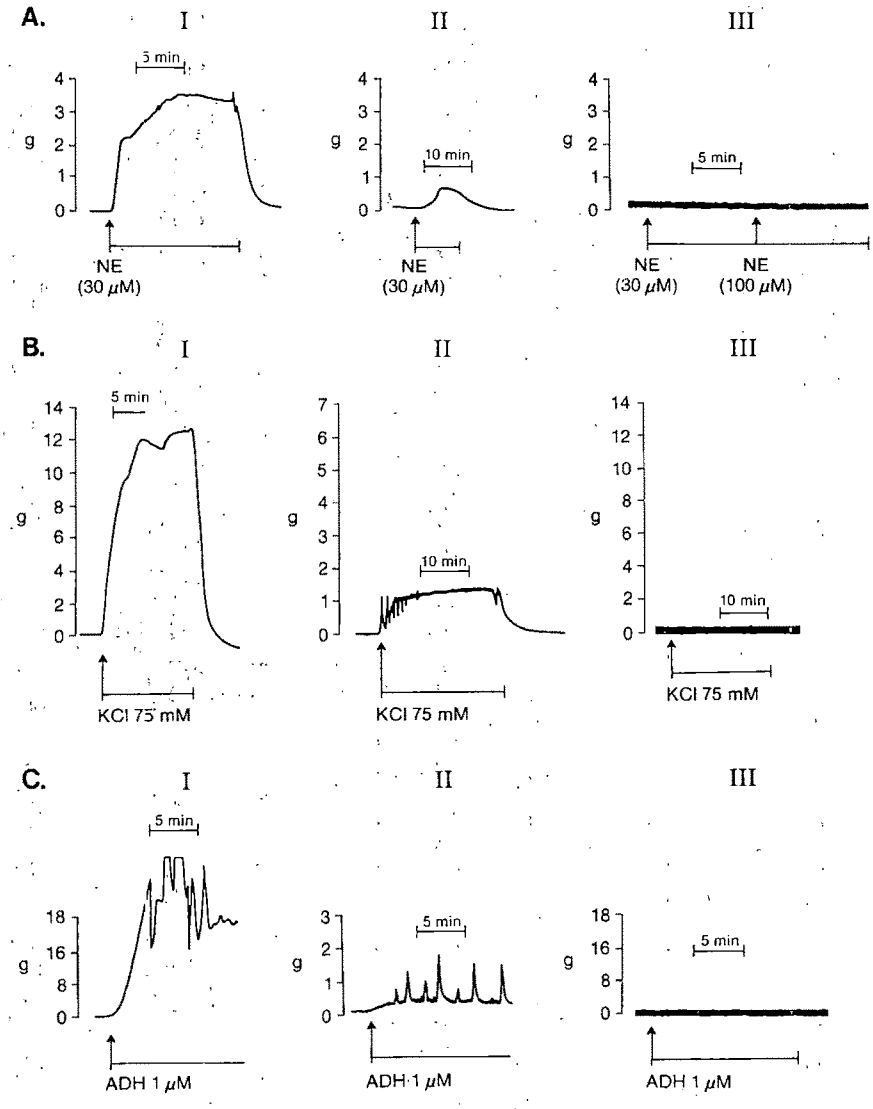


Fig. 1. Records of experiments showing effects of norepinephrine *(NE)*, potassium chloride *(KCl)*, and arginine vasopressin *(ADH)* in isolated uterine arteries from the various patient types (*I*, *II*, and *III*). Concentrations of constrictor agents produced maximum or near-maximum constrictions in control patients *(I)*.

appear to be any differences in the endothelial and smooth muscle layers of the arteries from type I (control) patients ($n = 3$) or type II ($n = 1$) or III (hemorrhaging) patients ($n = 2$).

Comment

During obstetric hemorrhage the patient is often given drugs to increase uterine tone. These drugs, including oxytocin, ergot alkaloids, vasopressin (Pitressin), and $PGF_{2\alpha}$, have vasoconstrictor action. The patients may also receive vasopressors such as ephedrine and phenylephrine. In this study isolated uterine arteries from patients experiencing obstetric hemorrhage demonstrated depressed or lack of reactivity to some of the same vasoconstrictor drugs used in the patient. To our knowledge this is the first report of such an altered reactivity in the isolated uterine vasculature of patients with obstetric hemorrhage compared with pregnant patients who undergo cesarean hysterectomy for other medical reasons.

This study involved only uterine arteries from pregnant patients, which eliminates the variability in results that may occur when pregnant and nonpregnant conditions are compared. We have previously shown that the norepinephrine or potassium chloride concentration that produced 50% of the maximal contraction and the maximal contractile response to norepinephrine or potassium chloride are similar in uterine arteries from pregnant and nonpregnant patients.[2, 3] However, other studies comparing pregnant and nonpregnant condi-

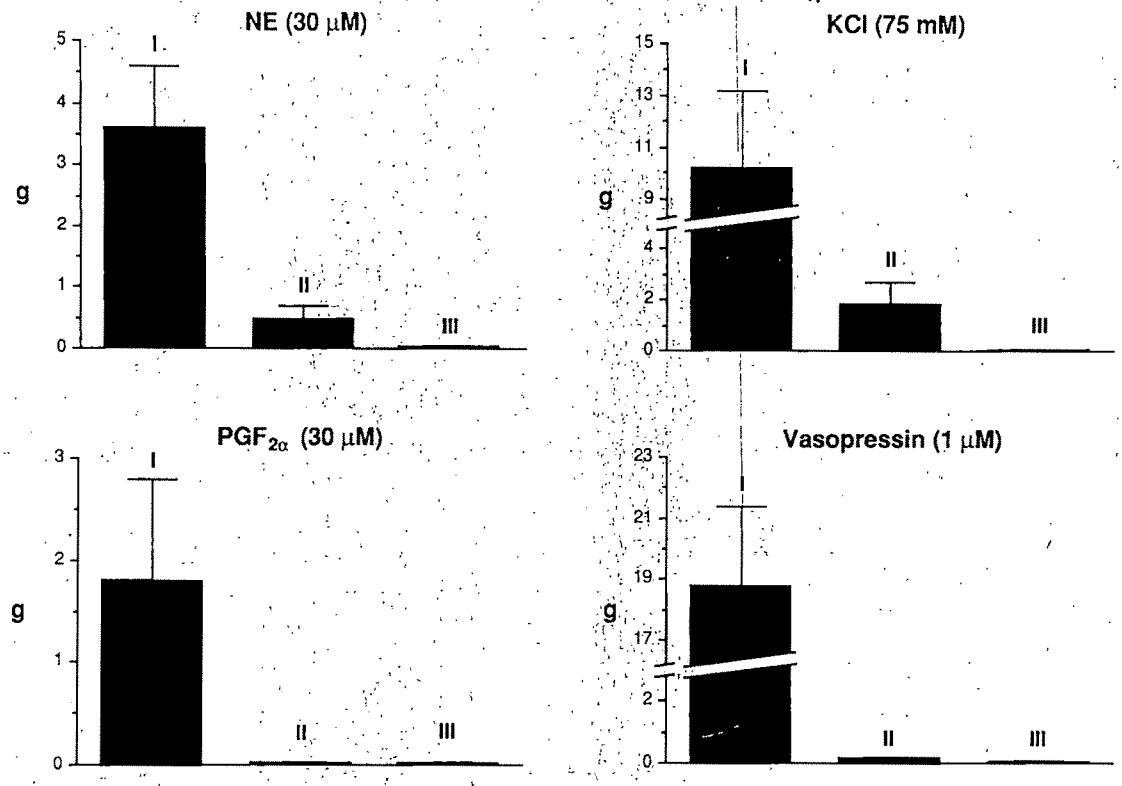


Fig. 2. Summary of results showing depressed constrictions induced by vasoconstrictors that act through various mechanisms in uterine arteries from patients with obstetric hemorrhage. Data were obtained from experiments similar to those shown in Fig. 1. Each *bar* represents constrictor response in grams (*g*) produced by vasoconstrictor and is mean ± SE of data from various patient types (*I*, $n = 9$; *II*, $n = 5$; *III*, $n = 2$).

tions show a variability in the constrictor responses to drugs such as norepinephrine, phenylephrine, or $PGF_{2\alpha}$ in uterine arteries from pregnant sheep,[13-17] rabbits,[18] and guinea pigs.[19]

An effort was made to determine the mechanism for the depressed reactivity in the uterine arteries from the patients experiencing hemorrhage. Agents were used that have been shown to produce vasoconstriction by different mechanisms such as activation of receptor-operated and/or voltage-dependent calcium channels as well as the release of calcium from intracellular storage sites. These various compounds produced no or only low constrictions. The results suggest that the depressed mechanism is common to all of the constrictors. Thus perhaps the contractile machinery itself is impaired.

It is possible that these uterine arteries from patients experiencing obstetric hemorrhage may have elevated levels of an endogenous vasodilator that is continuously released. We have previously reported that endothelium-derived relaxing factor production or release is increased in uterine arteries from normal pregnant patients when compared with that of nonpregnant patients.[4] However, in the uterine arteries from patients with hemorrhage, methylene blue (an inhibitor of the action of endothelium-derived relaxing factor or nitric oxide on soluble guanylate cyclase)[20] did not increase the baseline or enhance the subsequent response to norepinephrine to any greater extent than it did in normal pregnant uterine arteries. Furthermore, indomethacin, a cyclooxygenase inhibitor, did not increase the baseline or increase the constrictor response to norepinephrine, which indicates that an endogenous vasodilator prostaglandin was not continuously released or released with exposure to norepinephrine.

In other blood vessels the β_2-adrenergic receptor has been shown to mediate vasodilatation. Norepinephrine has a low affinity for the vasodilatory β_2-adrenergic receptor but a high affinity for the β_1-adrenergic receptor. Propranolol, a nonselective β-adrenergic receptor antagonist, was used to determine whether there was an increased affinity of the β_2-adrenergic receptor for norepinephrine or a change in the subtype from β_2 to β_1 in the uterine arteries that showed depressed norepinephrine-induced constrictions. Such changes would be reflected in a depressed norepinephrine-induced constriction. However, the response to norepinephrine was unaffected by the presence of propran-

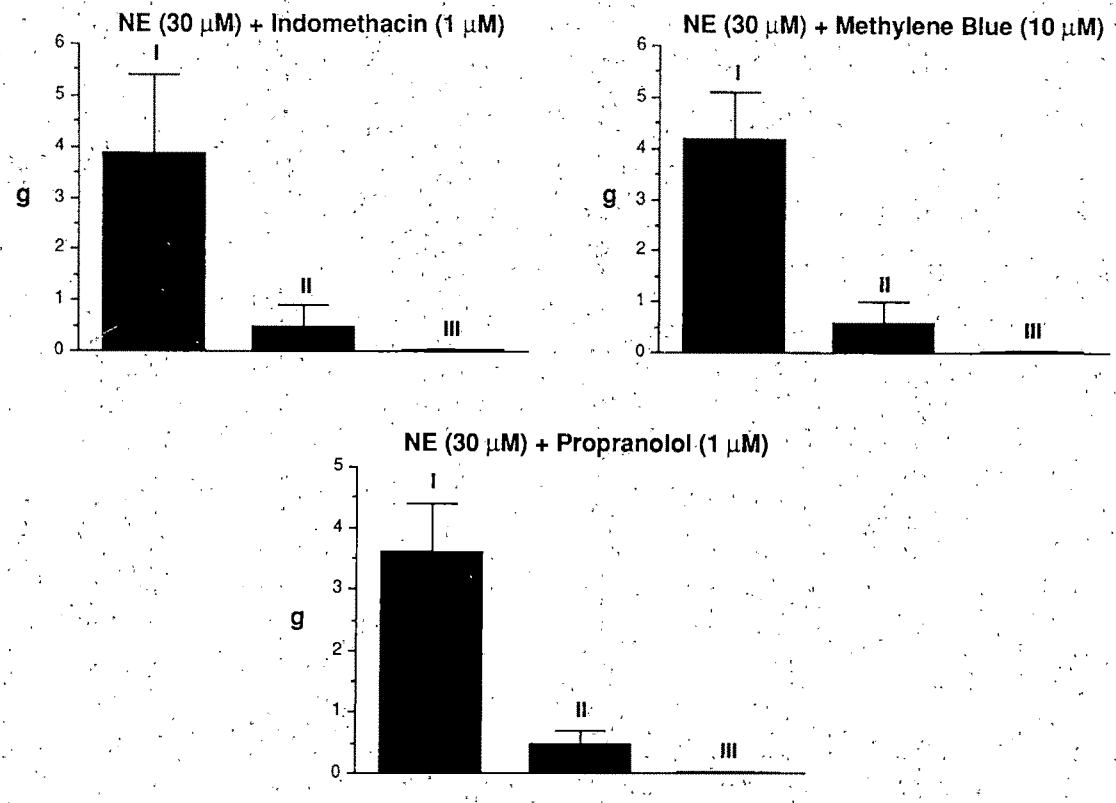


Fig. 3. Lack of effect of inhibitors of various vasodilator mechanisms. Norepinephrine (30 μmol/L) was administered for 15 minutes in absence and presence of inhibitor. Inhibitor was present for 30 minutes before norepinephrine was readministered. Each *bar* represents constrictor response to norepinephrine in grams *(g)* in presence of inhibitor (*I*, $n = 9$; *II*, $n = 5$; *III*, $n = 2$).

olol. Thus it appears that in the uterine arteries, as well as in other blood vessels, norepinephrine is selective for the β_1-adrenergic receptor and that there is no change in the β-adrenergic receptor subtype in the uterine arteries from patients with obstetric hemorrhage.

Numerous studies have shown a good correlation between cAMP concentrations in blood vessels and the degree of relaxation produced by stimulators of adenylate cyclase (e.g., isoproterenol). The basal levels of cAMP were not elevated in the uterine arteries from the patients with hemorrhage compared with those of uterine arteries from control pregnant patients. Hence our results exclude the possibilities that overstimulation of adenylate cyclase or dysfunctional adenylate cyclase or its regulatory G proteins (Gi or Gs) may be involved in the depression of contractility in the uterine arteries.

The mechanism for the depressed or lack of reactivity of the uterine arteries from hemorrhaging patients remains unclear. In a review of the charts of the patients, no risk factors could be found that were common to all of the hemorrhaging (types II and III) patients. Risk factors included causes of obstetric hemorrhage, presence of infection, development of hemorrhagic shock (volume of blood loss and fall in mean arterial pressure), parity, drugs, and associated clinical phenomena. Histologically, there did not appear to be any differences in the intimal or medial layers in the uterine arteries. Furthermore, the depressed or lack of reactivity could not be washed out from the isolated uterine vessels with time.

It is generally thought that obstetric hemorrhage is associated with a number of causes such as uterine atony, abnormal placentation, trauma during labor and delivery, and coagulation defects.[1] The results of this study, which used isolated uterine arteries from patients experiencing obstetric hemorrhage, suggest that these uterine arteries have lost their ability to constrict as a result of an unidentified mechanism. Failure of uterine artery constriction may contribute to uncontrollable obstetric hemorrhage.

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A reevaluation of estrogen status in postmenopausal women who smoke

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Hypoestrogenism in postmenopausal smokers has been suggested as the mechanism for the observed decreased risk of endometrial cancer and increased risk of osteoporosis. We have prospectively studied a well-matched group of smokers and nonsmokers and have evaluated their estrogen levels and compared them with existing data from the literature. We conclude that increased adrenal activity resulting in increased androgens, mainly androstenedione, is seen in postmenopausal smokers but that estrogen levels are not decreased. We hypothesize that in nonusers, unlike in users of estrogen, smoking is not associated with changes in estrogen levels and that other mechanisms must be responsible for the epidemiologic observations seen. (AM J OBSTET GYNECOL 1992;166:1444-8.)

Key words: Smoking, postmenopausal women, hormonal status

Recent studies have shown that postmenopausal women who smoke have a decreased risk of endometrial cancer and an increased risk of osteoporosis.[1,2] It has been assumed that the mechanism for these observations is profound hypoestrogenism, with levels of estrogen being lower in postmenopausal women who smoke. Our recent study[3] and data from others[4-8] suggest that among users of oral estrogen hepatic metabolism of estrogen is increased with smoking, which results in lower estrogen levels.[3-8] However, we hypothesize here that smoking in nonusers of estrogen does not result in lower estrogen levels, and therefore other mechanisms for the epidemiologic findings need to be entertained. To this end, we have prospectively studied a well-matched population of postmenopausal women not taking estrogen who were either smokers or nonsmokers and have evaluated their hormonal status. We have also compared and combined our data with the findings of other investigators in the literature.

Material and methods

Subjects. Thirty-eight postmenopausal women agreed to participate in this study in response to individual letters sent to members of a retirement community in the Los Angeles area. All women had been postmenopausal for ≥5 years. None had received hormonal replacement therapy, prednisone, cortisone, or anticoagulants for 6 months before the study. All patients were healthy and had no prior history of cancer or chronic illness. Seventeen women were nonsmokers, and 21 were chronic smokers who reported smoking an average of 19.9 cigarettes per day (Table I).

Protocol. Volunteers were studied in the morning after they had eaten their usual breakfast. Smokers did not smoke on the morning of the study before baseline testing (approximately 8 AM). Blood samples were drawn at baseline and 3 hours later. During these 3 hours smokers were instructed to smoke five of their usual brand of cigarettes. Sera obtained were separated and stored at −20° until assayed. Values for tar and nicotine content on brands of cigarettes were obtained from the United States Federal Trade Commission Report.[9]

Assays. All samples were assayed for estrone, estradiol, the estrogen conjugate estrone glucuronide, androstenedione, dehydroepiandrosterone sulfate (DHEAS), cortisol, sex hormone–binding globulin (SHBG), and non-SHBG-bound estradiol.

Estrone, estradiol, androstenedione, non-SHBG-bound estradiol, and SHBG levels were measured in serum by previously validated assays.[10-15] Estrone and androstenedione were measured by radioimmunoassay (RIA) after diethyl ether extraction and Celite chromatography. Estradiol was measured by RIA after extraction with ethyl acetate/hexane (4:6, vol/vol), by means of an iodinated estrone kit from Pantex (Santa Monica, Calif.). Tritiated estradiol was added to the aliquot of serum in which non-SHBG-bound estradiol was measured. All labeled and unlabeled estradiol bound to SHBG was precipitated. The remaining labeled unbound and non-SHBG-bound estradiol in the supernatant was counted and expressed as a percent of the total tritiated estradiol added. The resulting per-

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centage was multiplied by the serum estradiol to determine non-SGHB-bound estradiol in picograms/milliliter. SHBG was quantitated by determining its binding affinity to dihydrotestosterone in 0.1 ml of serum with the charcoal technique, which uses the temperature-dependent properties of proteins. The interassay coefficient of variation determined in pooled serum was 13% for estrone, 13% for estradiol, 12% for androstenedione, 10% for non-SHBG-bound estradiol, and 13% for SHBG.

Estrone glucuronide was measured in an aliquot of 0.6 ml of serum combined with an internal standard of approximately 1000 disintegrations/min tritiated estradiol glucuronide (Amersham Laboratories, Buckinghamshire, England; specific activity 12.6 Ci/mmol) and dissolved in 0.1 ml of 0.2 mol/L sodium phosphate buffer, pH 6.8, to monitor procedural losses, then incubated at 37° C for 30 minutes. Two milliliters phosphate buffer is added to the aqueous layer after extraction to remove unconjugated steroids. To each sample 500 U of β-glucuronidase (type VII-A from *Escherichia coli*, Sigma Chemical Company, St. Louis) in 0.1 ml of phosphate buffer was added and incubated 16 to 18 hours at 37° C. The hydrolyzed estrogens were extracted twice with 5 ml ethyl acetate/hexane (4:6, vol/vol). After the organic solvents were evaporated, the extract was reconstituted and the estrone was measured as previously described. All values are corrected for molecular weight. The sensitivity of the assay, defined as the smallest amount of estrone glucuronide per RIA tube that reduces the number of counts per minute of tritiated estrone glucuronide bound at zero mass by 2 SD, was 3.9 pg per tube or 45 pg/ml. Accuracy of the RIA was established as follows. Known quantities of authentic estrone glucuronide was added to aliquots of pooled serum. Linear regression analysis of the estrone glucuronide measured in pooled serum versus the amount of conjugated steroid added was done. A correlation coefficient of 0.99 was obtained. The specificity of the enzyme was 0.27% for conjugated sulfates. The interassay coefficient of variation for estrone glucuronide was 10%.

Serum DHEAS was determined by RIA as previously described.[16, 17] Cortisol was measured by RIA after its extraction from serum (0.01 ml) with ethyl acetate/hexane (1:1, vol/vol). The antiserum was raised against cortisol-3-(*O*-carboxymethyl)oxime-bovine serum albumin (ICN/RSL, Irvine, Calif.) and was used in conjunction with tritiated cortisol (DuPont NEN Research Products, Boston; specific activity 45 Ci/mmol). The antibody-bound cortisol was separated from the unbound with dextran-coated charcoal. The interassay coefficient of variation for DHEAS and cortisol were 16.4% and 12.1%, respectively.

The ratio, androstenedione/estrone, was calculated as a measure of aromatase activity.

Table I. Characteristics of volunteers population

	Nonsmokers	*Smokers*
No.	17	21
Age (yr)	72.3 ± 2.3	68.8 ± 3.9
Weight (kg)	62.9 ± 9.3	64.1 ± 7.1
Height (m)	1.59 ± 0.04	1.60 ± 0.07
Cigarettes (No./day)	0	19.9 ± 8.3
Tar (mg/day)	0	204 ± 155
Nicotine (mg/day)	0	14.9 ± 11.1

*Values are mean ± SD.

Statistical analyses. All hormonal measurements were transformed to logarithmic values to approximate normal distributions for statistical analysis. Group values are reported as geometric mean concentrations. Standard statistical methods of analysis of variance (and covariance) were used in the analysis of the results. All p values quoted are two-sided.

Results

The characteristics of the postmenopausal women sampled are shown in Table I. The mean age was 72.3 years for the nonsmokers and 68.8 years among smokers; this was not statistically significant. The groups were well matched for height and weight. Smokers averaged 19.9 cigarettes per day with an average tar of 204 mg per day and an average nicotine of 14.9 mg per day.

The geometric mean hormone levels of the nonsmokers and smokers are given in Table II. When smokers are compared with nonsmokers, estrone was 13% lower at baseline and 6% lower after smoking. These changes, however, were not statistically significant. Estrone glucuronide values were similar in smokers and nonsmokers, being only 5% and 4% greater before and after smoking. Estradiol changes were also not significant. They were 5% greater and 1% less before and after smoking. SHBG was 22% lower at 8 AM and 11% lower at 11 AM in smokers, but this also was not statistically significant. Bioavailable non-SHBG-bound estradiol was not significantly different in smokers and nonsmokers.

Androstenedione was 28% higher at 8 AM in smokers ($p = 0.13$) and 52% higher at 11 AM (after the smokers had smoked five cigarettes over a 3-hour period, $p = 0.011$). Nonsmokers showed a decrease in androstenedione over time (492 pg/ml at 8 AM vs 404 pg/ml at 11 AM, $p = 0.13$); the decline was much less in smokers (628 vs 614 pg/ml).

DHEAS was 42% higher at 8 AM and 62% higher at 11 AM in smokers than in nonsmokers, but these results did not achieve statistical significance. Cortisol was 3% lower at 8 AM in smokers than in nonsmokers but 19% higher at 11 AM (after smoking). This also was not statistically significant. As expected, cortisol showed a

Table II. Serum hormone levels in postmenopausal nonsmokers and smokers*

	Baseline (AM)				*After smoking (11 AM)*			
	Nonsmokers	*Smokers*	*(Smokers − nonsmokers)/ nonsmokers† (%)*	*p‡*	*Nonsmokers*	*Smokers*	*(Smokers − nonsmokers)/ nonsmokers† (%)*	*p‡*
No.	17	21			17	21		
Estrone (pg/ml)	38.7	33.7	−13	0.30	34.1	32.1	−6	0.71
Estrone glucuronide (pg/ml)	182	191	+5	0.62	190	198	+4	0.64
Estradiol (pg/ml)	15.1	15.8	+5	0.69	14.6	14.5	−1	0.95
SHBG (nmol/L)	158	123	−22	0.20	145	129	−11	0.56
Non-SHBG-bound estradiol (pg/ml)	4.48	5.96	+33	0.12	4.87	5.39	+11	0.51
Androstenedione (pg/ml)	492	628	+28	0.13	404	614	+52	0.011
DHEAS (μg/dl)	59.3	84.5	+42	0.20	54.8	88.7	+62	0.10
Cortisol (μg/dl)	16.1	15.7	−3	0.75	11.7	13.9	+19	0.16
Androstenedione/estrone	12.7	18.7	+47	0.026	11.9	19.1	+61	0.009

*Results shown are geometric means.

†Comparison is difference between smokers and nonsmokers as percent of nonsmokers.

‡Two-sided statistical significance levels.

marked decrease over a 3-hour period in nonsmokers (16.1 vs 11.7 μg/dl, $p = 0.017$); the decline was much less in smokers (15.7 vs 13.9 μg/dl). Smokers showed a 47% higher androstenedione/estrone ratio at baseline ($p = 0.026$) and a 61% higher ratio after smoking ($p = 0.009$).

The results shown in Table II remained essentially unchanged after adjustment for age, age at menopause, weight, height, and alcohol consumption.

Comment

The data presented here suggest that in postmenopausal women who smoke there is no alteration in serum estrogen levels if they are not receiving hormonal replacement. Our data suggest that the effect of smoking on serum hormone levels in healthy postmenopausal women is an increase in adrenal androgens (androstenedione, DHEAS). This is evident at baseline (androstenedione, 28% increase; DHEAS, 42% increase) and to a greater extent later in the morning after smoking (androstenedione, 52% increase; DHEAS, 62% increase). There is evidence for an increased level of cortisol in smokers compared with nonsmokers. The normal diurnal decline of cortisol is blunted in the smoking group.

There have been at least four other comparative studies of serum hormone levels in postmenopausal smokers and nonsmokers.[6-8, 18] Some details and results of these studies together with our results are given in Table III.

All studies that have looked at serum androstenedione have found elevated levels in smokers (mean increase at 20 cigarettes per day of 41%, $p < 0.0001$, Table III). This 41% mean value may be too high, because the biggest effect was seen in the study of Friedman et al.[7] and their smoking study subjects were close to menopause and 5 years younger than their nonsmoking subjects. This discrepancy will tend to exaggerate any difference between smokers and nonsmokers as androstenedione declines at menopause and with increasing age. When the results of Friedman et al.[7] are excluded, the mean increase in androstenedione in smokers is 37% ($p = 0.0002$).

Friedman et al.[7] and Khaw et al.[6] also found, as we did, that DHEAS levels are elevated in smokers; however, Key et al.[18] did not. There is no obvious reason for the discrepancy between these studies. The subjects described by Key et al.[18] tended to be samples later in the day than the subjects in the other studies, but we know of no reason why this should be of any importance. Key et al.[18] did provide some evidence for adrenal activation in smokers; however, urinary 11-hydroxyketosteroids were increased. When the combined data are considered, the mean increase in DHEAS at 20 cigarettes per day is 18% ($p = 0.059$, Table III). The results of Friedman et al.[7] may again be considered suspect for the reasons given above, and when their results are excluded, the mean increase in DHEAS in smokers is 14% ($p = 0.15$).

Cortisol was elevated in our smokers but only at 11 AM after smoking. The smokers reported by Friedman et al.[7] had elevated baseline levels. We have no explanation for this discrepancy. When all the available data are examined, changes in androstenedione, DHEAS, and cortisol all appear to be higher in smokers.

When the results are taken together, there does not

Table III. Results of previous studies of serum hormone levels in postmenopausal nonsmokers and smokers

	Reference								
	Friedman et al.[7] (1987)	*Key et al.[18] (1991)*	*Khaw et al.[6] (1988)*	*Longcope and Johnston[8] (1988)*	*Cassidenti et al. (1992)*	*Mean**	*Combined two-sided p*	*Mean†*	*Combined two-sided p*
Time of sampling	8 AM (fasting)	PM (nonfasting)	±8 AM (fasting)	?	8 AM (nonfasting)				
Nonsmokers									
No.	16	213‡	187	29	17				
Age (yr)	54	57	69	53	72				
Weight (kg)	68	24.0§	24.7§	62	63				
Smokers									
No.	9	48	46	12	21				
Age (yr)	49	57	66	51	69				
Weight (kg)	69	24.4§	23.9§	58	64				
Average No. of cigarettes/day	25	15	12	15	20				
Estrone	−2%	—	+10%	−1%	−13%	−3%	0.66	−4%	0.65
Estradiol	+27%	+5%	+8%	−12%	+5%	+11%	0.16	+5%	0.58
SHBG	—	—	+13%	—	−22%	−9%	0.54	−9%	0.54
Androstenedione	+74%	—	+25%	+32%	+28%	+41%	<0.0001	+37%	0.0002
DHEAS	+88%	−5%	+43%	—	+42%	+18%	0.059	+14%	0.15
Cortisol	+47%	—	—	—	−3%	+16%	0.006	—	—

*At 20 cigarettes per day.

†At 20 cigarettes per day but with results of Friedman et al.[7] excluded.

‡For estradiol, the numbers of subjects were 40 and 36 for nonsmokers and smokers, respectively.

§Kilograms per square meter.

appear to be any effect of smoking on estrone and estradiol in postmenopausal women who are not receiving oral estrogen. It has been suggested that catechol estrogen metabolism is enhanced in smokers[19]; however, if this was a significant finding, we would expect that it would have resulted in a difference in estrogen levels, an effect that has not been observed. That serum estrone glucuronide and SHBG levels were also not increased in smoking women suggests that the enhancement in hepatic activity, which occurs with smoking, occurs only in oral estrogen users.

Our results, however, show that the androstenedione/estrone ratio distinguishes smokers from nonsmokers more effectively than androstenedione alone. This is consistent with experimental data of nicotine-inhibiting aromatase activity.[20,21] Because aromatase activity is decreased by smoking, we would expect the estrogen status of smokers to be decreased. However, because adrenal activity is enhanced, particularly androstenedione, estrogen levels are similar in smokers and nonsmokers.

Therefore current results suggest that smoking increases adrenal androgen levels (androstenedione, DHEAS) and, at least transiently, cortisol increases as well. How do these results explain the epidemiologic observations of the markedly reduced risk of endometrial cancer[1] and the markedly increased risk of osteoporosis[2] in smokers? We hypothesize here that these observations are not based on changes in estrogen status but rather on alterations in other systems.

There is no evidence that cortisol has an influence on endometrial tissue, but there is some evidence that androgens can inhibit estrogen-mediated effects in the rat uterus.[22] In one study[22] it was shown that dihydrotestosterone inhibited estrogen-mediated rat uterine growth, and this effect appeared to be due to a dihydrotestosterone-induced reduction in estrogen-induced ribonucleic acid transcription rather than to any inhibition of estrogen receptor synthesis (as is true of progesterone). The only relevant epidemiologic data of androgen levels in endometrial cancer have been reported by Judd et al.[23] They found no reduction in androstenedione in endometrial cancer cases as would be expected if androstenedione antagonized the effect of estrone in endometrial tissue. Indeed, there is evidence that some patients with endometrial cancer have increased androgen levels, which may be related to hyperinsulinemia.[24] The obese insulin-resistant patient may in fact have an additional risk factor because insulin may stimulate endometrial aromatase activity.[25] Although androgens may still be inhibitory to estrogen action in the endometrium of normal-weight women who smoke, there are probably other mechanisms to explain the epidemiologic observations.

Reasons that smokers are at greater risk of osteoporosis may be due to the relative cortisol excess in smokers. Smoking itself may also have direct toxic (carbon monoxide, nicotine) effects on bone cells, thereby leading directly to osteoporosis. It is well known that smoking is toxic to other tissues; e.g., smoking influ-

ences oocyte atresia[26] and may impair sperm density, motility, and morphologic features.[27] However, increased androstenedione levels would tend to be beneficial to bone.

In conclusion, we suggest that estrogen levels are not decreased in nonusers of oral estrogen and therefore may not be implicated in the associated epidemiologic findings of a decrease in endometrial cancer and an increase in osteoporosis. Our findings and those of others reviewed here strongly suggest that there is increased adrenal activity in smoking women. This, as well as direct toxic effects of smoking, may be associated with the epidemiologic observations. More studies should be directed at these other mechanisms rather than at alterations in estrogen metabolism.

Addendum

In our patients we more recently measured 24-hour urinary estriol and estrone to assess whether estrogen metabolism may be altered (lowered 16-hydroxylase activity, thus favoring 2-hydroxylase), as suggested previously.[19] The 42% decrease in the estriol/estrone ratio described[19] suggested enhancement of the conversion to catechol estrogens; however, we found only a 6% decrease. The ratio was 1.028 in nonsmokers and 0.963 in smokers ($\dot{p} = 0.37$).

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Human fetal bilirubin levels and fetal hemolytic disease

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The development of secondary fetal anemia in association with maternal red blood cell alloimmunization requires hemolysis. In specimens obtained at the time of a clinically indicated cordocentesis, total and direct umbilical venous bilirubin was measured and the indirect umbilical venous bilirubin calculated in 43 antigen-positive and 30 control fetuses. Twenty-two (51%) of the antigen-positive fetuses had or subsequently developed severe anemia (hematocrit <30%). Umbilical venous total bilirubin ($r = 0.47$, $p = 0.0008$) and direct bilirubin ($r = 0.520$, $p = 0.04$) levels each rose with gestation. Indirect bilirubin did not vary significantly with gestation. Bilirubin was unrelated to hemoglobin. In contrast to the control fetuses, umbilical venous total bilirubin for antigen-positive fetuses was inversely related to hemoglobin ($r = -0.57$, $p < 0.0001$) independent of gestational age ($r = 0.53$, $p < 0.0001$) (multiple R of hemoglobin and gestational age for umbilical venous total bilirubin = 0.76, $p < 0.0001$). Eighteen of 22 (82%) fetuses in whom anemia developed had an umbilical venous total bilirubin ≥97.5 percentile compared with only eight of 21 (38%) fetuses in whom anemia did not develop ($p = 0.009$). In longitudinal study the umbilical venous total bilirubin frequently rose above normal weeks before the development of anemia. An umbilical venous total bilirubin >3 mg/dl represented the warning line. Fifteen of 16 (94%) fetuses in whom either severe antenatal anemia or significant postnatal hyperbilirubinemia developed had an umbilical venous total bilirubin >3 mg/dl. We conclude that the normal placental capacity for the transport of fetal bilirubin is exceeded in the face of enhanced fetal hemolysis. An elevated fetal bilirubin often precedes the development of antenatal anemia. The antigen-positive fetus with an elevated bilirubin is at high risk to develop anemia antenatally. (Am J Obstet Gynecol 1992;166:1449-54.)

Key words: Fetus, bilirubin, hemolytic anemia, alloimmunization, cordocentesis

Although the fetal response to maternal red blood cell alloimmunization depends on several factors, including the quantity of antibody bound to the fetal red blood cell and the rate of red blood cell production,[1] the development of fetal anemia ultimately requires hemolysis. In a preliminary study our unit observed that the normal fetal concentration of bilirubin exceeded that of the neonate.[2] This suggested that the placental capacity for fetal bilirubin clearance was limited. The purposes of this investigation were to determine (1) the concentration of fetal bilirubin across gestation, (2) whether antigen-positive fetuses of alloimmunized women have higher bilirubin concentrations than control fetuses, and (3) whether a high bilirubin concentration preceded the development of fetal anemia.

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Material and methods

Cordocentesis was performed in 73 pregnancies with referral to the University of Iowa Fetal Diagnosis and Treatment Unit. Forty-three antigen-positive fetuses at risk for hemolytic anemia were sampled (95 specimens) to determine the severity of their disease as described previously.[1] The remaining 30 fetuses were sampled once for a variety of indications (most commonly maternal red cell or platelet alloimmunization) and shown to be free of disease.

Laboratory measurements performed in the central hospital laboratory included total bilirubin, direct bilirubin, and a complete blood cell count. The indirect bilirubin was calculated. Total and direct bilirubin (mg/dl) were measured in serum by a commercially available colorimetric assay (Boehringer-Mannheim) on an autoanalyzer (Hitachi 737). Hemolyzed samples were excluded. The complete blood cell count was performed with an autoanalyzer (Technicon H-1) on blood anticoagulated with ethylenediaminetetraacetic acid. Because of the limited sample volume, not all tests could be performed on each occasion.

The technique for cordocentesis has previously been detailed.[3] Briefly, a 22-gauge spinal needle was held in the ultrasonographic plane of a 5 MHz sector transducer with a fixed-needle guide (CIVCO, Kalona, Ia.).

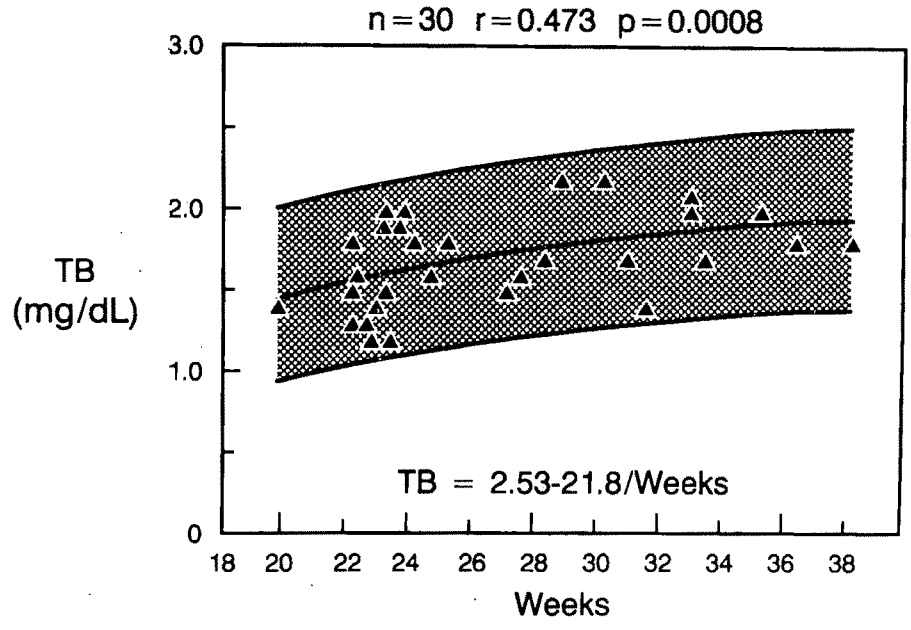


Fig. 1. Prediction interval (95%) for umbilical venous serum total bilirubin *(TB)* in healthy, antigen-negative fetuses ($n = 30$). There was no relationship between bilirubin and hemoglobin in this group.

The umbilical vein was preferred over the umbilical artery to minimize the risk of a procedure-related bradycardia.[3] Pancuronium (0.3 mg/kg estimated fetal weight) was administered intravenously whenever fetal movement was felt to pose a risk.

All records were maintained prospectively in a computerized data base whose veracity was confirmed on a regular basis. The results are presented as the mean ± SD unless otherwise stated. One hundred eleven of 125 (90.2%) specimens were from the umbilical vein. Only the first measurement from each antigen-positive fetus was included in the search for a relationship between bilirubin and both hemoglobin and gestational age. Because the small number of arterial specimens precluded an accurate comparison of arterial and venous samples controlling for gestational age, the statistical analyses presented here were limited to specimens from the umbilical vein. The 95% prediction interval of bilirubin for control fetuses was based on the regression obtained using bilirubin as the dependent variable and gestational age as the independent variable. Subsequent bilirubin measurements from antigen-positive, not transfused, fetuses were graphed to reveal the natural history. An analysis of covariance was used to compare the bilirubin slopes of antigen-positive and control fetuses. Receptor-operated curves were applied to determine the umbilical venous total bilirubin concentration most predictive of a fetal or neonatal hemolytic complication. Other analyses included independent t test, stepwise multiple regression, and cross tabulation. A p value ≤ 0.05 was considered to indicate a significant difference.

Results

The mean gestational age of fetuses at risk for hemolytic anemia was 24.9 ± 3 weeks and ranged from 20 to 36 weeks. Twenty-two (51%) developed a hematocrit <30% and were considered candidates for transfusion therapy.[4] The mean gestational age of the control group was 27 ± 5 weeks and ranged from 20 to 38 weeks.

Umbilical venous total bilirubin ($r = 0.47$, $p < 0.0008$, $n = 30$) (Fig. 1) and direct bilirubin ($r = 0.52$, $p = 0.04$, $n = 15$) for the control group rose progressively with advancing gestation and were unrelated to the fetal hemoglobin. Indirect bilirubin did not change significantly with gestation (mean 1.4 ± 0.3 mg/dl, $n = 15$).

In contrast to the control fetuses, umbilical venous total bilirubin from antigen-positive fetuses was inversely related to hemoglobin ($r = -0.57$, $p < 0.0001$) (Fig. 2) independent of gestational age ($r = 0.53$, $p < 0.0001$) (multiple R of hemoglobin and gestational age for umbilical venous total bilirubin = 0.76, $p < 0.0001$). There was no relationship between hemoglobin and gestational age in the antigen-positive fetuses ($r = 0.05$, $p = 0.75$). Although there was a significant relationship between umbilical venous total bilirubin and gestational age in both groups, they were different (Fig. 3). Umbilical venous total bilirubin in antigen-positive fetuses rose at a rate significantly greater ($p < 0.01$) than that expected for the normal gestational increment. A similar association was also observed for both the direct and indirect bilirubin concentrations and hemoglobin and gestational age (Table

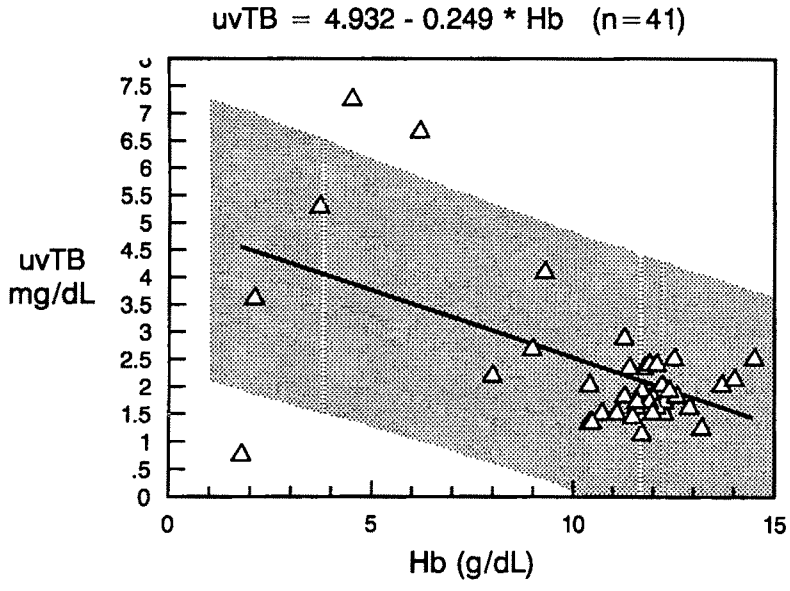


Fig. 2. Relationship between total umbilical venous bilirubin *(uvTB)* and hemoglobin *(Hb)* in antigen-positive fetuses. The higher the bilirubin concentration, the lower the fetal hemoglobin. Note that total bilirubin of 3 mg/dl approximates hemoglobin of 8 gm/dl (two missing values).

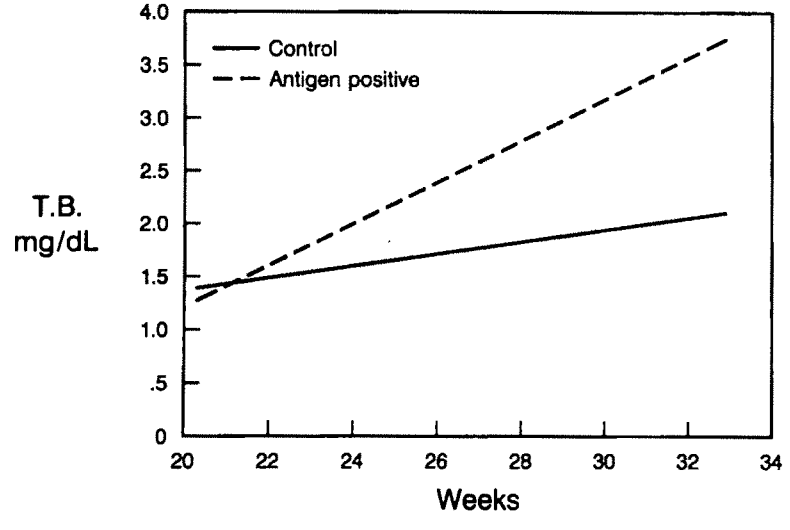


Fig. 3. Regression lines for umbilical venous total bilirubin *(T.B.)* and gestational age in control and antigen-positive groups. Antigen-positive fetuses have steeper slope. This demonstrates that severity of fetal hemolytic disease was progressive.

I). Because the relationships between indirect and direct bilirubin for hemoglobin in antigen-positive fetuses were similar to that for umbilical venous total bilirubin, we have focused on the latter because of the larger sample size.

The umbilical venous total bilirubin exceeded the 97.5 percentile in 18 of 22 (82%) of antigen-positive fetuses who developed a hematocrit <30%. In eight of 10 fetuses with two or more samples, the elevation preceded the development of anemia by several weeks (Fig. 4). In contrast, only eight of 21 (38%) of fetuses who did not develop severe anemia had an umbilical venous total bilirubin above the 97.5 percentile at any time before delivery ($p = 0.009$ from fetuses who required transfusion) (Fig. 5). Overall, the positive predictive value of a high umbilical venous total bilirubin for the development of severe fetal hemolytic anemia was 69% (18 of 26), and the negative predictive value of a low umbilical venous total bilirubin for the development of severe anemia was 76% (13 of 17).

We sought to determine whether the clinical application of the umbilical venous total bilirubin measurement could be further simplified by the identification of a warning line. An umbilical venous total bilirubin

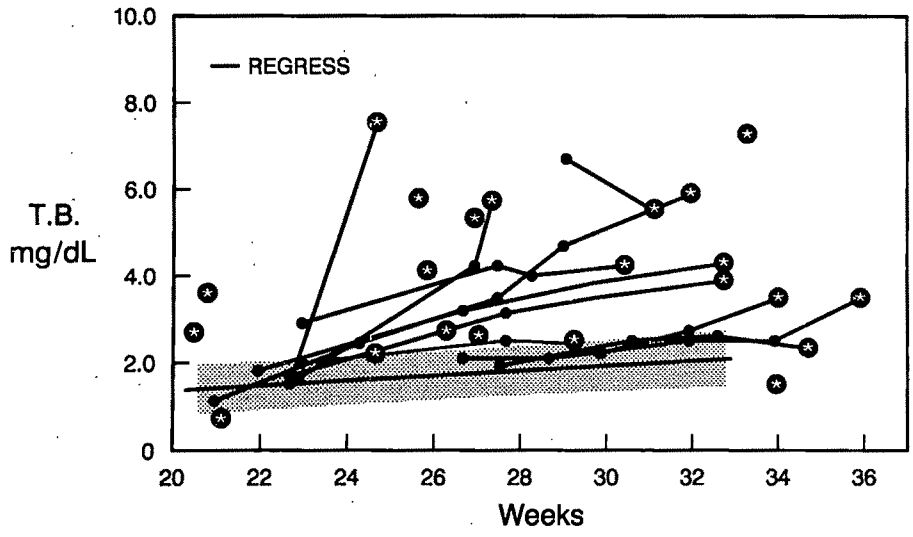


Fig. 4. Serial umbilical venous total bilirubin *(T.B.)* concentrations in fetuses who developed severe hemolytic anemia. Last sample *(asterisk)* indicates that hematocrit had declined below 30%. Single samples indicate that initial hematocrit was <30% first time bilirubin was measured. *Shaded area* reflects 95% prediction interval for normal range. Note that elevated umbilical venous total bilirubin often preceded development of anemia severe enough to require transfusion.

Table I. Relationship between bilirubin, hemoglobin, and gestation in first sample from antigen-positive fetuses

Bilirubin group	*No.*	*R*	*p*	*r*	*p*
Total	43	0.76	<0.0001		
Hemoglobin				−0.57	<0.0001
Weeks' gestation				0.53	<0.0001
Direct	39	0.68	<0.0001		
Hemoglobin				−0.53	0.0001
Weeks' gestation				0.46	0.0007
Indirect	39	0.74	<0.0001		
Hemoglobin				−0.56	<0.0001
Weeks' gestation				0.52	<0.0001

>3 mg/dl exceeded the 97.5 percentile for all gestational ages. Six fetuses already had severe anemia when first sampled: five of the six (83%) had an umbilical venous total bilirubin ≥3 mg/dl. The umbilical venous total bilirubin ultimately rose above 3 mg/dl in another nine fetuses whose initial hematocrit was >30% and whose initial umbilical venous total bilirubin <3 mg/dl. Seven of these nine (78%) developed severe hemolytic anemia antenatally. The two fetuses who did not develop anemia antenatally experienced severe hyperbilirubinemia postnatally (one required two double-volume exchange transfusions and the other required 10 days of phototherapy). Fetuses whose umbilical venous total bilirubin before transfusion was <3 mg/dl had lower reticulocyte counts than fetuses whose umbilical venous total bilirubin was ≥3 mg/dl (10.5% ± 4.2% vs 24.7% ± 12.6%, $p = 0.0003$).

We previously described four hematologic patterns predicting the severity of fetal hemolytic disease on the basis of fetal hematocrit, direct Coombs test, and reticulocyte count.[1] These patterns were prospectively assigned to each of the 14 fetuses who had or developed an umbilical venous total bilirubin >3 mg/dl. Two fetuses were assigned pattern 1 (not at risk to develop severe anemia antenatally), three were pattern 2 (intermediate risk for anemia), and nine were pattern 3 (high risk). Each of three with pattern 2 and eight of the nine (89%) with pattern 3 developed severe hemolytic anemia. Of the remaining three fetuses, two experienced significant hyperbilirubinemia postnatally (as noted in the above paragraph). One of those two fetuses had been assigned pattern 1 and the other pattern 3.

Comment

We have examined the effect of gestational age and fetal hemolytic disease on the concentration of umbilical venous bilirubin. Normal fetal umbilical venous total and direct bilirubin (not shown) rose similarly with advancing gestational age. On the basis of the scattergram, it is likely that with a larger sample size we would have also observed a rise in the indirect bilirubin concentration. Although the number of measurements performed on specimens from the umbilical artery was inadequate to assess placental clearance of bilirubin di-

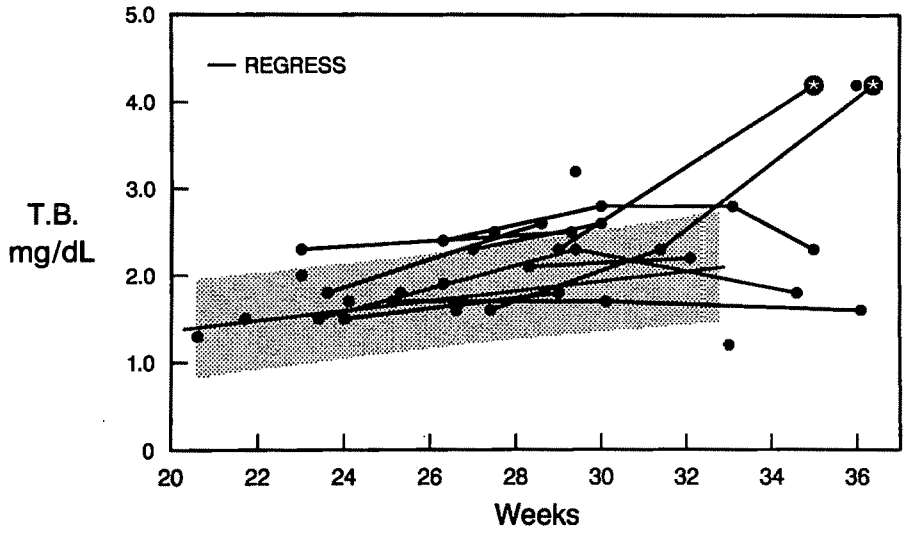


Fig. 5. Serial umbilical venous total bilirubin *(T.B.)* measurements of fetuses whose hematocrit remained above 30%. *Shaded area* indicates 95% prediction interval for normal umbilical venous total bilirubin. The two fetuses marked by *asterisk* developed significant postnatal hyperbilirubinemia.

rectly, the observation that normal neonatal bilirubin is much lower[2] than that of the near-term fetus suggests that placental capacity to transport the normal production of fetal bilirubin to the mother is relatively low.

Moise et al.[5] reported a single alloimmunized fetus whose serum was icteric and speculated that it reflected hemolysis.[5] In antigen-positive fetuses, there was an inverse relationship between hemoglobin and bilirubin independent of gestational age. The high concentration of bilirubin in the sera of antigen-positive fetuses was not likely secondary to decreased placental clearance because there was a significant inverse correlation between fetal hemoglobin and bilirubin in the antigen-positive group that was absent in the control group. This suggests that the greater the quantity of red blood cell hemolysis, the lower the hemoglobin.

The use of fetal blood instead of amniotic fluid for the evaluation of maternal red cell alloimmunization is a new concept not yet widely accepted. This unit has previously demonstrated that the antigen-positive fetus at risk for the development of severe hemolytic anemia can be prospectively identified on the basis measurements of fetal hematocrit, reticulocyte count, and strength of the direct Coombs test.[1] On the basis of these three tests, the antigen-positive fetus can be assigned to one of four hematologic risk patterns. The higher the pattern number, the greater the risk of the fetus developing a hematocrit <30%. The current investigation suggests that the addition of umbilical venous total bilirubin measurement to that battery of tests may be of value, especially in instances where the assigned pattern suggests the fetus is at no or low risk for the development of severe hemolytic anemia before birth. In our experience, only one of five fetuses with pattern 2 will become severely anemic antenatally. In the current series each of the three fetuses with both pattern 2 and an umbilical venous total bilirubin level >3 mg/dl developed severe hemolytic anemia. And although no fetus with both pattern 1 and an umbilical venous total bilirubin level >3 mg/dl developed severe anemia, one of the two did require 10 days of phototherapy postnatally.

Not all fetuses who became anemic had a high bilirubin level. This may reflect, in part, variability of placental bilirubin transport. However, there is an additional explanation. We have previously demonstrated that a sizable group of antigen-positive fetuses who become anemic do so gradually in association with a low reticulocyte count and a weakly positive direct Coombs test.[1] In the current investigation, fetuses whose umbilical venous total bilirubin level was <3 mg/dl before transfusion had significantly lower reticulocyte counts than fetuses whose level was ≥3 mg/dl. This suggests that the degree of hemolysis in some anemic fetuses is relatively low but that they fail to respond to red blood cell destruction with an adequate erythropoietic response. This observation would also explain why the amniotic fluid Δ optical density 450 of anemic fetuses is not always elevated.

In conclusion, the ability of the human placenta to clear fetal bilirubin is limited. Because of this limitation, fetal hemolytic disease is associated with fetal hyperbilirubinemia. The higher the bilirubin concentration, the lower the hemoglobin concentration. An elevated

fetal serum bilirubin indicates that the fetus is at high risk to develop severe hemolytic anemia antenatally. It may also provide additional information regarding the risk of postnatal hyperbilirubinemia.

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Origin of cervical collagenase during parturition

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Cervical biopsy specimens were obtained under standard conditions from the posterior lip of the uterine cervix in 105 patients. A significant increase of collagenase activity was observed during parturition as determined with an assay with iodine 125–labeled native triple-helical collagen type I as the substrate. The collagenase was not likely to originate from cervical fibroblasts because in situ hybridization failed to detect synthesis of the specific procollagenase messenger ribonucleic acid. However, migration of polymorphonuclear leukocytes into the cervical stroma occurred on onset of labor, and an antibody specific for human leukocyte collagenase that did not cross react with fibroblast collagenase revealed the presence of the enzyme in the granules of polymorphonuclear leukocytes and subsequently in the extracellular matrix of the cervix. Therefore it is likely that the cells critically involved in collagen degradation during cervical dilatation are not resident fibroblasts but rather polymorphonuclear leukocytes emigrating from blood vessels. (Am J Obstet Gynecol 1992;166:1455-60.)

Key words: Pregnancy, cervix, cervical dilatation, parturition, collagenase

A significant drop of hydroxyproline concentration in the cervix at the end of pregnancy has been frequently described[1-4] and attributed to increased collagenolysis, especially during childbirth.[5-7] However, no serial determinations of collagenase activity have been carried out in the cervix during childbirth. Kitamura et al.[8] found evidence for increased collagenolysis at the time of delivery compared with cervix samples from nonpregnant women. Ito et al.[9] determined the activity of a PZ-peptidase (4-phenylazobenzyloxycarbonyl-Pro-Leu-Gly-Pro-D-Arg) that may play a role in collagen degradation and found a marked increase in its concentration during pregnancy. All of these investigations must be viewed cirtically because small peptides were used as substrates to measure collagenolytic activity. Rath et al.[10] quantified the true collagenase activity by using native, triple-helical collagen as a substrate. The highest activity was measured immediately after parturition. No differences in collagenase and other protease activities were detected between samples from pregnant women during the first trimester and from nonpregnant patients. In the current study sequential examination of true collagenase activity at various times during the course of pregnancy and, in particular, during delivery was carried out. The source of cervical collagenase, which had not been previously determined, was also examined. Fibroblasts in many types of tissue are, of course, capable of synthesizing collagenase, but there are indications that other cell types, e.g., polymorphonuclear leukocytes, may be critically involved in the process of cervical dilatation during parturition.[11]

The aim of the current study was to establish the time course of the appearance of active collagenase in the cervix during parturition and to determine its cellular origin.

Material and methods

The design of the study was submitted to the appropriate ethics committees and accepted. Patients were informed in detail about the aims and the procedures of the study and gave their written consent. Cervical biopsies were performed under standardized conditions at various times during pregnancy and in nonpregnant patients by excising tissue with a scalpel from the posterior lip of the cervix at the 6 o'clock position to a depth of 5 to 10 mm. Samples of 50 to 200 mg wet weight were obtained. For the determination of collagenase activity and for in situ hybridization the tissue was deepithelialized, washed in ice-cold sterile saline solution, and deep frozen within 2 to 3 minutes in liquid nitrogen. The specimens were stored at −70° C in polypropylene tubes (Nunc) wrapped in aluminum foil until further examination. A total of 49 samples was available for collagenase extraction or in situ hybridization (Table I). An additional 115 specimens were procured under the same conditions for immunohistologic studies. They came from nonpreg-

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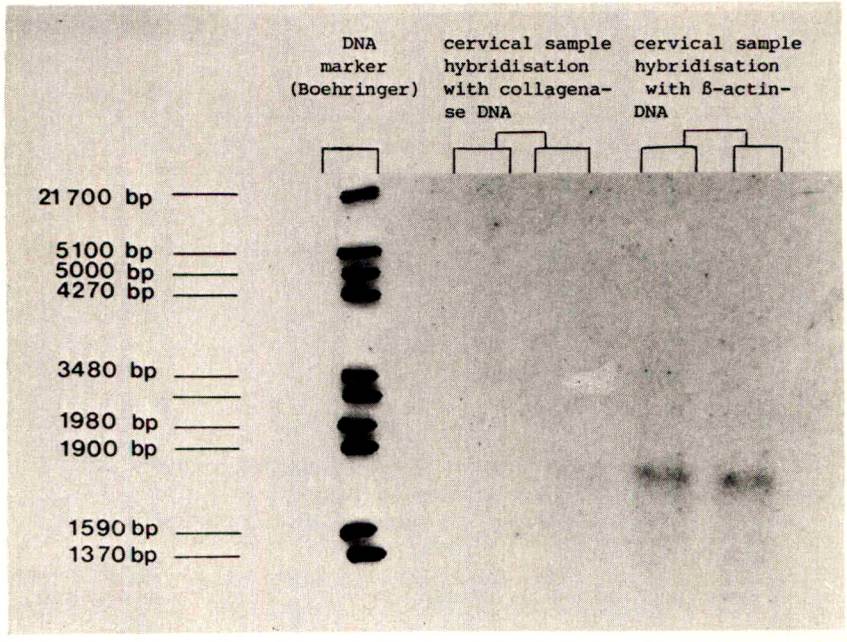


Fig. 1. Northern blot of mRNA extracted from cervical tissue and hybridized to phosphorus 35–nick translated complementary deoxyribonucleic acid *(DNA)* of β-actin or human fibroblast collagenase. Positive signal was obtained with probe for β-actin but not with that for collagenase, regardless of kind of cervix tissue.

Table I. Collagenase activity in extracts of cervical tissue

Origin of specimen	*No.*	*Collagenase activity*		*Significance (Wilcoxon–Mann-Whitney test)*
		Median ± SEM	*Range*	
Nonpregnant, premenopause	9	25.4 ± 1.4	12.4-31.7	
First trimester	10	26.3 ± 2.4	19.1-44.4	
Third trimester (no labor)	4	28.6 ± 3.8	14.7-31.6	$p < 0.05$
Latent phase of labor (cervical dilatation 2-3 cm)	5	27.0 ± 9.3	13.2-63.4	$p < 0.01$
Active phase of labor (cervical dilatation 6-8 cm)	8	40.0 ± 4.6	30.4-62.3	
Immediately post partum	13	29.1 ± 4.7	15.2-67.6	

Data represent nanograms of collagen degraded by the extract of 100 mg (wet weight) tissue.

nant, premenopausal patients (n = 20) and from pregnant patients during the first trimester (n = 20), during the third trimester (before elective cesarean section, no labor, unripe cervix; n = 15), in the latent phase of labor (cervical dilatation 2 to 3 cm, normal delivery; n = 15), in the active phase of labor (cervical dilatation 6 to 8 cm, normal delivery; n = 15), and immediately after delivery (n = 30). These tissues were not deepithelialized but were washed in ice-cold saline solution. They were then fixed in acid formalin and embedded in paraffin.

Active collagenase was extracted with neutral buffer and then assayed with native, triple iodine 125–labeled collagen as substrate.[10] In situ hybridization was carried out exactly as described by Scharffetter et al.[12]

The original clone (designated Coll K4) for human fibroblast collagenase was kindly donated by P. Herrlich, Karlsruhe, Germany. It was subcloned in a GEM3 vector (GEM3 plasmid, 2867 kb, Promega, Madison, Wis.). The plasmid GEM3 Coll K4 was cleaved by *Ava* I and *Hin*dIII (Boehringer Mannheim, Mannheim, Germany) and the linearized plasmids were purified by sodium acetate precipitation and phenol-chloroform extractions. Antisense and sense ribonucleic acid (RNA)

probes were obtained with sulfur 35–uridine 5′-triphosphate and by incubating the GEM3 Coll K4 *Ava* I vector with SP6 RNA polymerase and the GEM3 Coll K4 *Hin*dIII vector with T7 RNA polymerase, respectively. Total RNA was extracted from some cervix tissues by the isothiocyanate method of Davis et al.[13] and hybridized in Northern blots to nick translated deoxyribonucleic acid for β-actin or human fibroblast collagenase (matrix metalloproteinase 1). Normal dermal fibroblasts were grown in Dulbecco's minimal essential medium containing 10% fetal calf serum and exposed to 25 ng/ml tumor necrosis factor-α for 18 hours. Messenger RNA (mRNA) extracted from these cells was hybridized in Northern blots to ^{35}S-labeled antisense and sense RNA for β-actin or human fibroblast procollagenase. For immunofluorescence studies the tissue in paraffin was sectioned and paraffin was removed by passage through descending alcohol concentrations. The sections were rinsed in water or phosphate-buffered saline solution, followed by incubation with the immunoglobulin G fraction of an antiserum to human leukocyte collagenase. This antibody had previously been characterized and shown to react with human leukocyte collagenase but not with human fibroblast collagenase.[14, 15] The sections were incubated with this antibody at room temperature for 2 hours, rinsed extensively with phosphate-buffered saline solution, and stained with a fluorescein isothiocyanate–conjugated monoclonal antibody to rabbit immunoglobulin G in the dark for 2 hours at room temperature. After careful washing, the sections were embedded in glycerol–phosphate-buffered saline solution and immediately evaluated. We used the Wilcoxon–Mann-Whitney test for comparison of results, and the level of significance was set at $p \leq 0.05$.

Results

Cervical softening during parturition is likely to involve breakdown of collagens, which, in turn, requires the presence of specific collagenases. We therefore attempted to directly demonstrate such enzyme activity in cervical tissues during delivery and from controls. Biopsy specimens from a total of nine nonpregnant and 40 pregnant patients could be evaluated. Samples were available from pregnant patients in the first and third trimesters, during two stages of cervical dilatation, and immediately post partum. Active collagenase could be extracted from all specimens (Table I). There was great variation between individual samples and no statistically significant difference of the medians of collagenase activities between biopsy specimens from nonpregnant patients, patients in the first or third trimester (without labor), and in the latent phase of labor with early stages of cervical dilatation. However, collagenase activity rose substantially and significantly ($p < 0.01$) in the final stage of dilatation (6 to 8 cm) compared with the first trimester and dropped immediately post partum.

Although the presence of active collagenase was thus established, the source of the enzyme has yet to be determined. It may originate in the cervical tissue, either from resident fibroblasts or from migratory polymorphonuclear leukocytes. Fibroblasts would be expected to synthesize the enzyme in situ, whereas polymorphonuclear leukocytes are known to have granules that contain collagenase along with other proteases. The activity of fibroblasts can be evaluated by examining the production of specific mRNA for fibroblast collagenase, whereas the collagenase from polymorphonuclear leukocytes is best demonstrated by a specific antibody.

Our results with in situ hybridization did not demonstrate the presence of a messenger for fibroblast collagenase. This could not be attributed to method inadequacies for the following reasons: RNA extracted from the tissue reacted in Northern blots with a complementary deoxyribonucleic acid for β-actin (Fig. 1). This shows that RNA in the specimens was largely intact and had not been destroyed by the extraction procedure. Also, RNA extracted from tumor necrosis factor-α–stimulated fibroblasts reacted in Northern blots with the specific ^{35}S-labeled antisense RNA probe (not shown). There appears to be a genuine lack of collagenase messenger activity in cervical fibroblasts during cervical dilatation, and therefore the collagenase extractable from the tissue is not likely to originate from these cells.

Polymorphonuclear leukocytes are known to contain collagenase, among other vesicular enzymes, and are thus probably the source of the cervical collagenase. Collagenase was indeed easily detected as granular fluorescence within polymorphonuclear leukocytes in specimens from nonpregnant patients and from pregnant women during the first and third trimesters. At the end of pregnancy and at the onset of labor there was accumulation of polymorphonuclear leukocytes in the capillaries and small veins (Fig. 2). Massive leukocyte infiltration of cervical connective tissue occurred at the onset of labor and at the beginning of cervical dilatation (Fig. 3). At later stages there was degranulation of the polymorphonuclear leukocytes; the granules contained and later shed collagenase (Fig. 3). Finally, there was a weak fluorescent staining of the collagen fibers (Fig. 4).

Comment

The uterine cervix derives its tensile strength and firmness from collagen, which is the predominant protein of the extracellular matrix of this tissue. Therefore

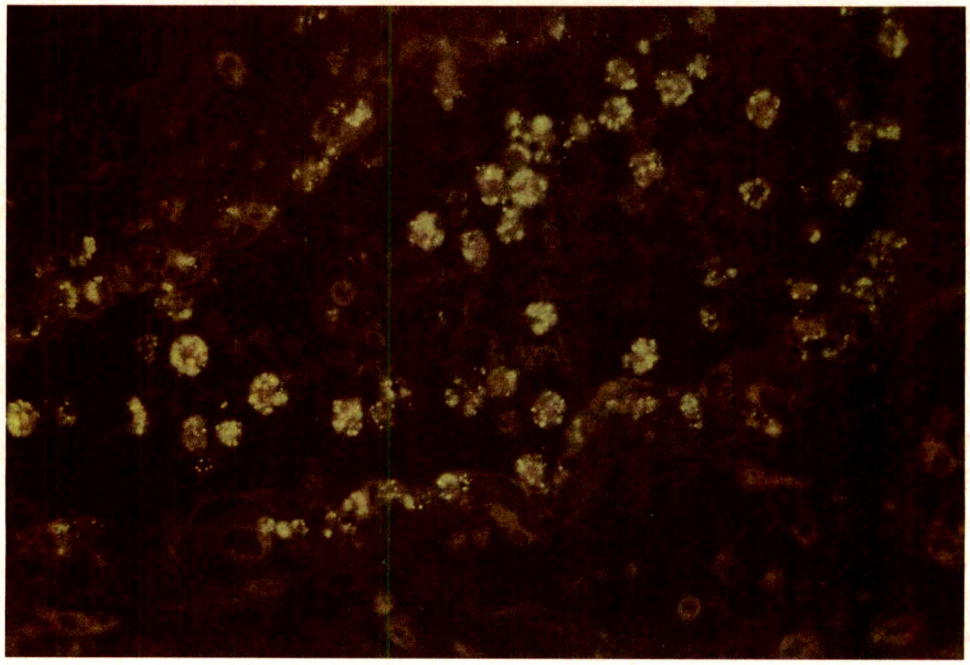


Fig. 2. Demonstration of leukocyte collagenase in cervical tissue by immunofluorescence, in third trimester without labor. Massive accumulation of polymorphonuclear leukocytes in small vein at end of pregnancy. (Original magnification ×630.)

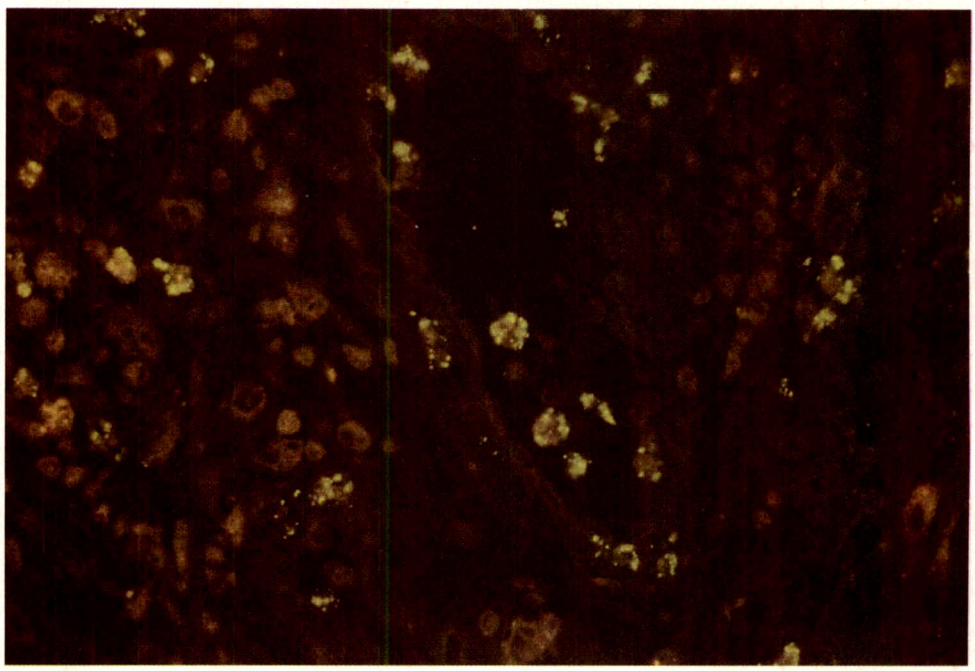


Fig. 3. Demonstration of leukocyte collagenase in cervical tissue by immunofluorescence, in latent phase of labor, with cervical dilatation 2 to 3 cm. Infiltration of cervical stroma by polymorphonuclear leukocytes with collagenase-containing granules. (Original magnification ×630.)

alterations in these biophysical parameters during labor and cervical dilatation are likely to involve changes of the organization of the collagen fibrils. Early experiments by von Maillot and Zimmermann[4] and by Kleissl et al.[16] have demonstrated changes in solubility of collagen during cervical dilatation and a decrease of hydroxyproline concentration of the cervix during parturition. These data have been confirmed and extended by several investigators[4, 17, 18] and correlate well with histologic observations that show rarification of colla-

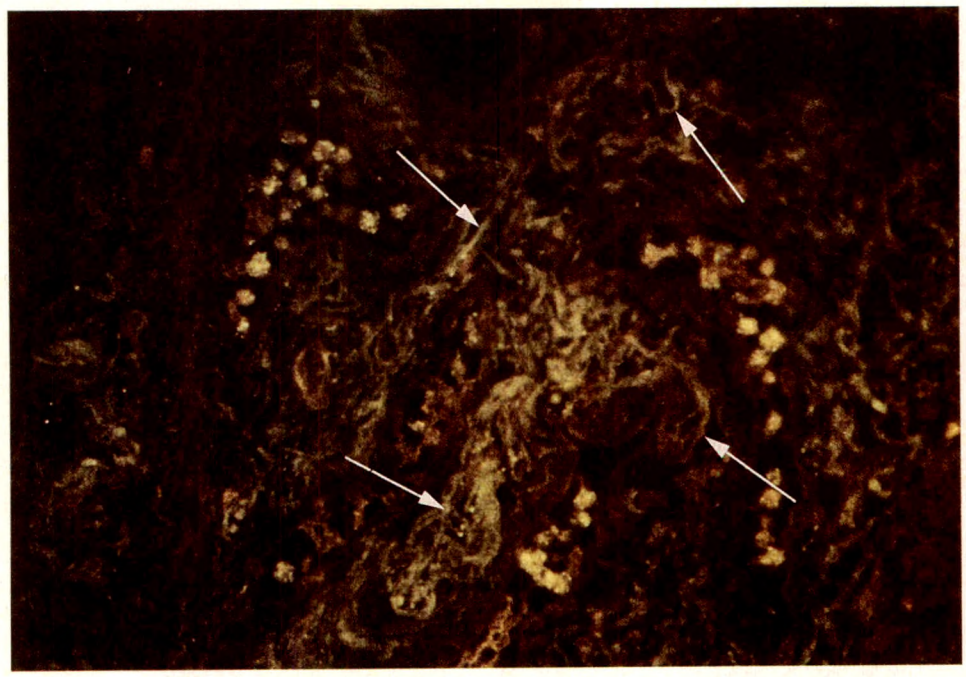


Fig. 4. Demonstration of leukocyte collagenase in cervical tissue by immunofluorescence, in active phase of labor, with cervical dilatation 6 to 8 cm. Some polymorphonuclear leukocytes are still visible within veins and capillaries, but in these late stages of labor there is additional faint staining of collagen fibrils *(white arrows)*. (Original magnification ×400.)

gen fibrils during labor by both light and electron microscopy.[11, 19]

Solubilization and degradation of collagen require attack by collagenase as the initial step. Cervical collagenase has been isolated[20] and increased collagenolytic activity has been demonstrated immediately post partum in humans[18] but not in some other species such as the guinea pig.[19] The current investigation provides data for the presence of active collagenase during pregnancy and labor. Other proteases are known to be present in the extracts of cervical tissue. These enzymes are not likely to interfere with the collagenase assay because the collagen was resistant to at least a tenfold excess of trypsin over substrate (unpublished data). The assay probably underestimated the amount of collagenase protein present in the cervical tissue because some of the enzyme may not be activated, may be inhibited, or may not have been extractable. The experiments were designed to determine the time of appearance of active collagenase in the tissue and thus the time of collagen breakdown. The data establish that maximal activity occurs transiently immediately before parturition.

It has to be emphasized that no conclusion can be drawn from our findings that the increase of collagenase activity occurs exclusively during the active phase of labor. Indeed, there is no significant increase during the active phase as compared with the latent phase of labor. The quite wide ranges in the latent phase are grounds for the assumption that the increase of collagenase activity in cervical tissue occurs much earlier, presumably with the onset of labor.

The source of cervical collagenase was then determined. Because fibroblasts and smooth muscle cells are known to be capable of producing collagenase, it was assumed that these cervical cells were the source of the enzyme during labor.[21, 22] The availability of two crucial reagents permitted us to study this issue in some detail. Because cells of the fibroblast type do not store collagenase but rather synthesize the enzyme on activation, it was possible to use a specific probe to study the expression of the corresponding mRNA during labor. However, this messenger mRNA could not be found in the cervical sections examined. Because this cannot be attributed to method inadequacies, it must be concluded that fibroblast cells are not likely to contribute a major portion of the collagenase observed. The conspicuous presence of polymorphonuclear leukocytes in the extravasal matrix of cervical connective tissue during parturition had already led Junqueira et al.[11] to surmise a connection between these cells and the process of cervical softening. Our investigation of the involvement of polymorphonuclear leukocytes was facilitated by the availability of a well-characterized antibody that had been shown[14] to react with leukocyte collagenase but not with fibroblast collagenase. The immunofluorescence data clearly demonstrated, concomitant with progressive cervical dilatation, that an accumulation of collagenase-containing leukocytes in

small vessels occurs, followed by an extravasation of these cells, degranulation and release of collagenase protein, and finally degranulation of collagen fibrils of the extracellular connective tissue matrix. Although this experimental approach can only demonstrate the presence of the enzyme protein and does not yield information on its enzymatic activity, it appears likely that this is indeed the enzyme that causes collagen breakdown during normal cervical dilatation. Further studies are required to elucidate the trigger mechanisms that cause accumulation, extravasation, and degranulation of polymorphonuclear leukocytes under these circumstances.

We thank Mrs. Cornelia Platte for technical assistance.

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Acute genital injury in the prepubertal girl

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In an effort to develop guidelines for the management of acute genital injuries in prepubertal girls, we categorized 32 cases by the object that allegedly caused the injury: straddle injuries, nonpenetrating injuries, penetrating injuries, and torque injuries. Using these categories and the anatomic features of symmetry and/or hymenal transection, we determined that the most dangerous injuries were the penetrating injuries that were symmetric and transected the hymen; in this series these were all the result of sexual assault. Future studies are needed to determine if these unique injuries can be managed with less physical and psychosocial trauma to the young patient. (Am J Obstet Gynecol 1992;166:1461-6.)

Key words: Genital injury, prepubertal girls, sexual abuse

Genital trauma in the prepubertal girl is often relatively minor, but it is frequently anxiety provoking to all involved individuals. With increased public and physician awareness of childhood sexual abuse, surgical specialists are more frequently being called on to assist in the management of these injuries.[1] Unfortunately there are no published guidelines on how to evaluate and/or manage genital injuries in girls. Most authors describing perineal injuries in children have confined their remarks to serious external genital injuries of the male child and/or impalement injuries.[2-4]

A retrospective review of 32 prepubertal girls with acute genital trauma was undertaken. The purposes of this case study are to bring to the surgical consultants' attention the psychosocial issues that are involved with these injuries and to establish guidelines for their care. Parents or primary care providers are frequently concerned as to whether sexual abuse has occurred. Certainly the process of evaluation and management of the wound can contribute to further physical and emotional trauma for the abused patient. Guidelines are developed that incorporate aspects of the history and characteristics of the wound and address the following issues: Were the viscera above the external genitalia likely to be injured? Could the wound be managed conservatively, or should the child be taken to the operating room for an examination while she is under anesthesia? Did the history given correlate with the type of wound observed?

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Methods and material

Thirty-two children with primary external injuries confined to the perineum were studied. The injuries caused acute bleeding and/or a painful outcry to the extent that the child was brought for emergency care. Patients whose bleeding was attributed to a genital pathophysiologic process other than trauma were excluded. Children whose primary presenting injury involved another area of the body yet who had a secondary genital wound were also excluded. Two children (cases 27 and 28) denied injury in spite of perineal bleeding and the presence of perivaginal lacerations and/or abrasions. They were placed in the penetrating category because their denial of trauma in light of gross wounds signaled the possibility of sexual abuse. Because estrogen thickens and increases the distensibility of vulvar tissues, only children ≤10 years old were included and none had entered puberty; the mean age was 6.33 years.

Demographic, historic, evaluation, management, anatomic, and follow-up data were gathered from the medical records (Table I). All of these children were examined by one author (S.F.P.), and 21 had photographs available for review.

Injuries were categorized by definition of the object that allegedly caused the injury. Straddle injuries were defined as falls onto smooth, horizontal, elongated narrow objects such as bicycle bars. Nonpenetrating injuries were caused by falls onto short, wide, and frequently irregular objects that were unlikely to penetrate above the pelvic floor. Penetrating injuries were associated with objects long and narrow enough to penetrate the pelvic floor; no stab or gunshot wounds were included. Torque injuries were complex injuries of the perineum caused by crush and torsion of the pelvic floor.

In an attempt to ascertain whether certain types of injuries should make the clinician suspicious of sexual

Table I. Acute genital injury in 32 prepubertal girls

Case No.	*Age (yr)*	*Race*	*History*	*Object*	*CPS*	*Symmetric or asymmetric*	*Hymen transected*	*Area*	*OR*	*Follow-up*
Straddle injuries										
1	3	H	Fall	Cabinet door	No	A	No	2	Yes	No
2	4	H	Fall	Bathtub rim	No	A	No	2	No	No
3	4	B	Fall	Fence	No	A	No	2	No	Yes
4	5	W	Jumped onto	Board	No	S	No	2	Yes	Yes
5	6	B	Accident	Bicycle bar	No	S	No	2	Yes	No
6	6	B	Accident	Bicycle bar	No	S	No	2	No	No
7	7	B	Accident	Bicycle bar	Yes	A	No	2	No	No
8	7	W	Accident	Bicycle bar	No	A	No	2	No	No
9	9	W	Accident	Bicycle bar	No	S	No	2	No	No
Nonpenetrating injuries										
10*	1	B	Fall	Bicycle part	Yes	S	No	2	Yes	Yes
11	3	H	Fall	Bicycle part	Yes	A	No	3	Yes	Yes
12	3	H	Fall	Tree stump	No	S	Yes	1	No	Yes
13	3	W	Fall	Toy	No	A	No	2	No	Yes
14	5	B	Fall	Shoe	Yes	A	No	3	No	No
15	5	B	Insertion	Doctor's finger	No	S	Yes	1	No	No
16*	6	H	Fall	Bicycle part	Yes	A	Yes	1	No	Yes
17	6	H	Kicked	Shoe	Yes	A	No	2	Yes	Yes
18	7	W	Jumped onto	Toy	No	S	Yes	1	No	Yes
19	7	W	Fall	Stool	Yes	A	No	2	No	No
20*	7	W	Fall	Toy	No	A	No	2	Yes	No
21	8	B	Fall	Tree stump	No	A	No	2	No	Yes
22	8	W	Fall	Stool	No	A	No	2	No	Yes
Penetrating injuries										
23	6	B	Rape	Penis	Yes	S	Yes	1	Yes	Yes
24	6	B	Rape	Penis	Yes	S	Yes	1	No	Yes
25	8	W	Jumped onto	Metal rod	No	S	No	1	Yes	Yes
26	8	B	Rape	Penis	Yes	S	Yes	1	Yes	Yes
27	8	B	Denied injury	?	Yes	S	Yes	1	No	No
28	10	B	Gonorrhea	?	Yes	S	Yes	1	No	No
29*	10	H	Rape	Penis	Yes	S	Yes	1	No	Yes
30	10	W	Rape	Penis	Yes	S	Yes	1	Yes	Yes
Torque injuries										
31	2	H	Accident	?	No	A	No	2	Yes	No
32	3	H	Crushed	Car	Yes	A	No	2	Yes	Yes

CPS, Children's Protective Services or other agency investigated case for sexual abuse; *OR*, case managed with patient under anesthesia; *W*, white; *H*, Hispanic; *B*, black; *S*, symmetric; *A*, asymmetric. For area: *1*, Wound entered vagina; *2*, primary wound confined to vestibule or labia minora; *3*, primary wound remained outside labia minora or vestibule.

*Children seen initially in other emergency centers and referred to S.F.P. for further and/or follow-up care.

abuse, the data were analyzed by comparing the anatomic findings with both the cause of the injury and the history. The Fisher exact test was used to test differences between proportions, and the Student *t* test was used to test differences between two means.

Results

The majority of these children were seen in the emergency center at Texas Children's Hospital, a referral children's hospital. The ethnic background of the subjects reflected the general population served: blacks, 40% ($n = 13$); whites, 31% ($n = 10$), and Hispanics, 28% ($n = 9$). Four children were initially managed at another facility and referred for follow-up care (cases 10, 16, 20, and 29); Two of these children received sutures for relatively minor superficial wounds that did not transect the hymen, and two others had transections of the hymen but did not undergo an examination under anesthesia.

Children's Protective Services or the police were involved with 15 patients: eight solely because of the genital location of the injury, five because of the child's allegations of sexual abuse, and two (cases 27 and 28) because of other concerns about abuse. In case 27 the patient denied any type of injury in spite of a fresh laceration extending from the posterior forchette into the vagina through an extremely attenuated hymen, suggesting both acute and chronic penetration (Fig. 1). In case 28 the patient was a retarded girl who, in addition to the acute genital injuries, had gonorrhea cultured from the vagina.

To determine if the eight children with no substantiation of abuse other than the genital injury were referred for investigation of childhood sexual abuse because they were younger (less verbal) and/or because of emergency room personnel bias, which would lead them to suspect sexual abuse in certain ethnic subsets, the age and race of these eight girls were analyzed.

These children were younger, with a mean age of 5.1 years versus 6.33 years for the group as a whole, and seven of the eight were of a minority background. Nevertheless, with the Student *t* test there was no statistical difference of age or of the racial distribution of these patients other than the racial distribution of the injury category from which that case originated. There was a trend for minority children to undergo more investigations for sexual abuse than white children.

The objects that allegedly caused the injuries are listed in Table I. The two torque injuries involved motor vehicles. The patient in case 31 was 2 years 9 months old and was thrown from a motorcycle. In case 32 the patient was a 3-year-old girl who was crushed between a building and a car; she sustained six fractures of the pelvis and a massive ischiorectal hematoma that dissected into the retroperitoneal space before rupturing through the vestibule and creating a massive perineal laceration.

The anatomic variables that were analyzed were the following (Fig. 2): (1) Was the wound symmetric or asymmetric? (2) Was the hymen transected? (3) Was the wound confined to the external genitalia? (4) Was the wound relatively minor or was it severe?

There were 16 symmetric and 16 asymmetric lesions. When a symmetric injury transected the hymen, there was a very strong possibility that the injury was caused by sexual abuse (seven out of 10 patients). Because of this association, symmetric injuries and ones that transected the hymen were analyzed in more detail.

Four of nine straddle injuries were symmetric, but none of them transected the hymen. Four of the 13 nonpenetrating injuries were symmetric, and three of these transected the hymen. These three wounds apparently were "direct hits": In case 12 the patient slid down a tree trunk onto a protruding irregular stump (Fig. 3); in case 15 the patient was being evaluated to rule out rectal bleeding when the emergency room physician's gloved finger inadvertently entered the vagina of the girl; in case 18 the patient jumped into a wading pool and landed on a plastic toy.

None of the three symmetric nonpenetrating injuries that transected the hymen acutely widened the vaginal introitus (Fig. 3), and the apex of the laceration was visible in the lower aspect of the vagina. The seven patients with symmetric penetrating injuries caused by rape all had a widened vaginal introital diameter (Figs. 1 and 4) when they were seen in the emergency center. In three of these injuries (cases 24, 27, and 28) attenuation of the hymenal tissue contributed to the widened diameter (Fig. 1).

In 16 children in whom the cause of the injury was known to be either a straddle or a nonpenetrating object (16/22) and who were hemodynamically stable, initial emergency room management consisted of reassurance, keeping her quiet and supine, and applying ice packs and a pressure dressing to the perineum. This ultimately resulted in decreased swelling and pain and gained the child's cooperation so that the wound and perineum could be copiously lavaged. It was then possible to determine the full extent of injury without a need for anesthesia.

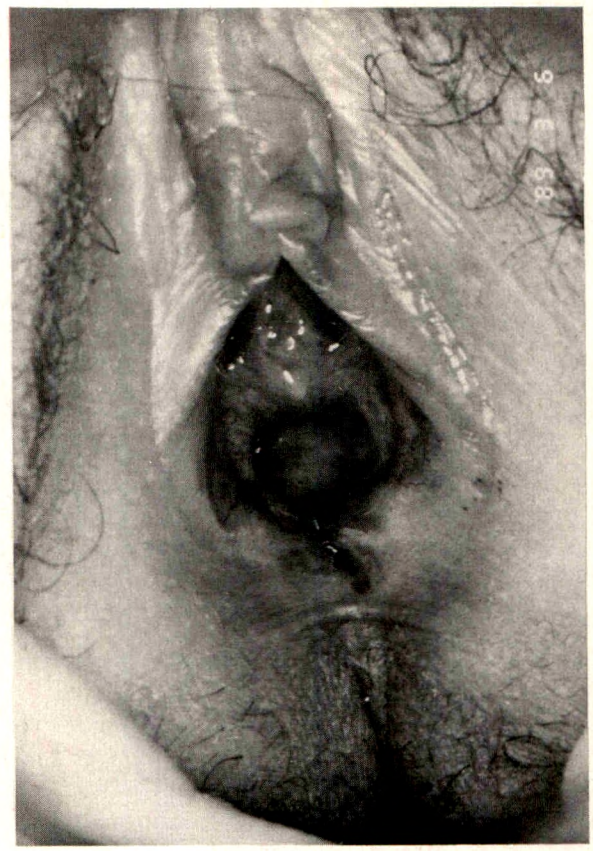


Fig. 1. In case 27 the patient was 8 years old with profuse vaginal bleeding, adamant denial of injury, and multiple social risk factors for abuse. Note attenuation of posterior hymenal tissue which is suggestive of chronic penetration.

Six of the 22 patients in whom the cause of injury was known to be either a straddle or a nonpenetrating object required an examination while they were under anesthesia. These six children were younger than the group as a whole, but there was not a statistical difference in their ages when the Student *t* test was used. They also remained uncooperative and had very hemorrhagic wounds (cases 1, 4, 5, 10, 17, and 20). The wounds were ultimately found to be relatively minor, requiring simple cauterization of a bleeder in three cases, placement of one suture in two, and approximation of a partially severed perineal body in one.

The most serious injuries in this series were three rape survivors (cases 23, 26, and 30), one accidental impalement (case 25), and two motor vehicle accident victims (cases 31 and 32). In all six of these girls an extensive injury was suggested preoperatively. The wound extended either into the upper vagina or into

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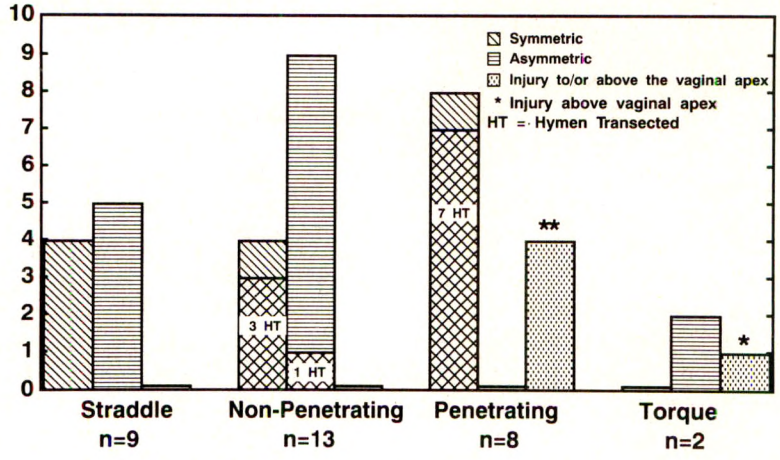


Fig. 2. Anatomic variables by cause of injury. *Bars* indicate whether wound was symmetric *(first bar)* or asymmetric *(second bar)* and whether injury extended to/or above vaginal apex *(third bar)*; three injuries *(asterisk)* extended above vaginal apex, and 11 injuries included hymenal transection *(HT)*.

the perineal tissues beyond what could be visualized by an external examination.

In three of these patients there was injury above the vaginal apex: In case 25 there was perforation of the rectum, cul-de-sac, and small bowel in multiple sites; in case 26 a vaginal laceration extended through the cul-de-sac into the abdominal cavity; in case 32 a massive hematoma extended into the right retroperitoneal space.

None of the three children with injuries above the vaginal apex had signs of higher abdominal or pelvic injury when first seen in the emergency center. In cases 26 and 32 the patients had extensive visible perineal injuries and were taken for operative repair, at which time the higher, more extensive injuries were detected. One patient (case 25), who had severe visceral injuries, did not complain of abdominal pain until several hours after being admitted and observed for what was thought to be a superficial penetrating injury to the perineal body. She was taken to the operating room after an acute abdomen developed.

Diverting colostomies were performed in three cases. In cases 25 and 32 they were performed to maximize healing of bowel injuries and/or to diminish fecal soiling of the perineal injuries, which were debrided, drained, and allowed to close by secondary intent. In case 23 the patient underwent a colostomy in preparation for primary closure of a fourth-degree perineal body laceration that was 5 days old when first seen. All three had excellent anatomic results.

All three rape survivors in whom operative intervention was required had vestibular lacerations that extended through a distinct rim of hymenal tissue, high into the vagina along the vaginal wall. Two patients (cases 26 and 30) also had another extensive laceration in the apex of the vagina, which extended from one lateral vaginal fornix in a curvilinear fashion, posterior to the cervix, and into the opposite vaginal fornix; this laceration extended into the abdominal cavity in one patient (case 26).

No endoscopic studies were performed unless the child was under anesthesia. Vaginoscopy, with the pediatric cystoscope, was performed on six of the 12 children who required an anesthetic for evaluation of their wounds. Because the hymen was intact in case 25, that impalement injury was the only vaginal wound that was unexpectedly discovered. The three sexual assault survivors who had endoscopic studies performed had obvious vaginal injuries that did not require vaginoscopy for discovery.

In this series there was only one patient (case 32) in whom cystoscopy was performed; that child had pelvic fractures and suspected urethral injury. However, the pelvic fractures were not discovered until she underwent an examination while she was under anesthesia and a bladder catheter was already in place.

Proctoscopy was performed on two girls while they were under anesthesia: In case 25 a rectal perforation was identified just inside the rectal verge by digital examination, and no higher perforation was identified by proctoscopy; in case 32 there were no rectal or colon perforations.

Although large vaginal lacerations were closed primarily, very few of the perineal injuries reported in this series required primary closure and/or suturing for hemostasis. In the majority of cases requiring operative intervention the wound was debrided and allowed to close by secondary intent, particularly if it was a sagittal laceration confined to the mucosa.

When the perineal body was transected, it was pri-

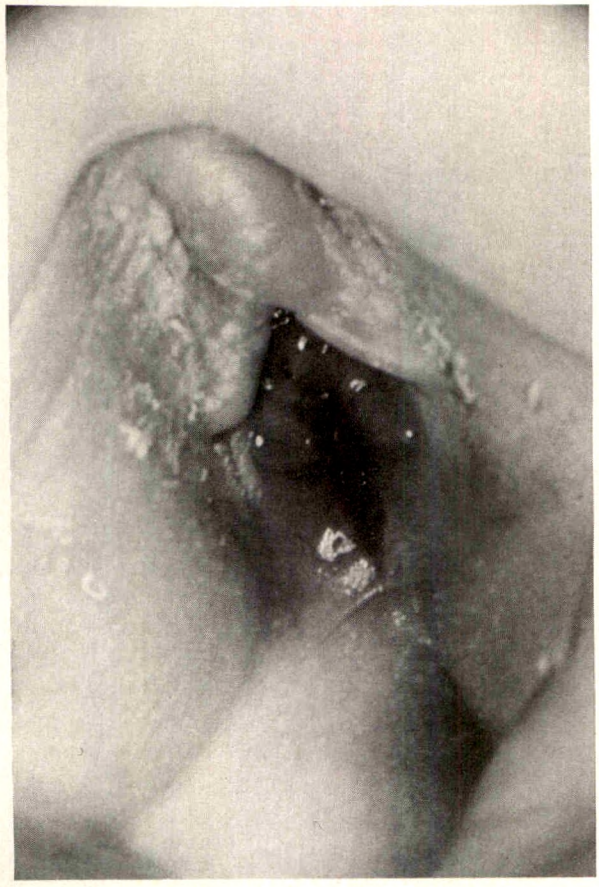


Fig. 3. In case 12 a 3-year-old girl slid down a tree trunk and lacerated the posterior fourchette, hymen, and 1 cm of the lower vagina on a smooth knuckle of tree stump.

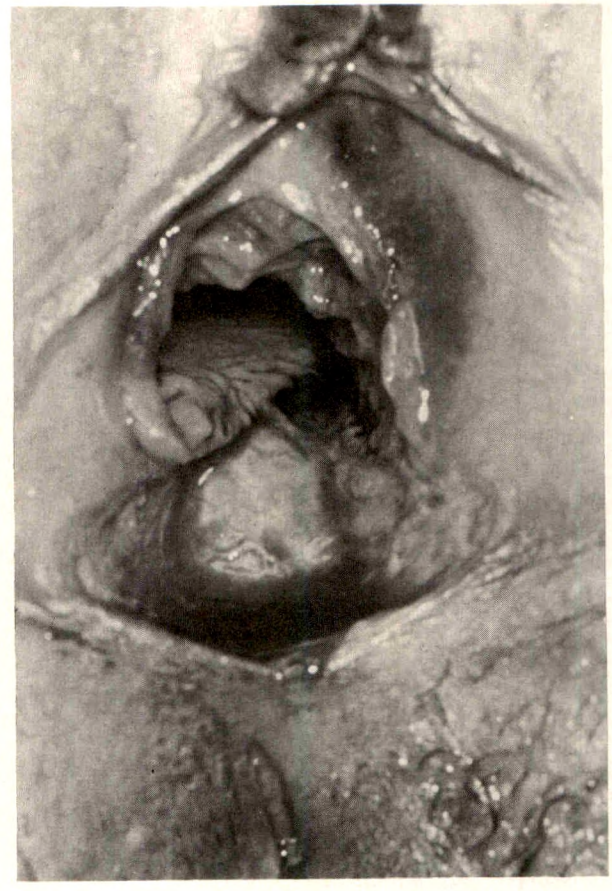


Fig. 4. In case 26 an 8-year-old girl was a survivor of stranger rape. She had avulsion of the vaginal apex with herniation of small bowel.

marily closed in two patients (cases 4 and 23) and allowed to close by secondary intent in three patients (cases 25, 31, and 32). The success in the latter three cases could be attributed to the fact that other injuries kept these children immobilized for a sufficient amount of time for secondary repair to occur.

Nineteen children were seen at follow-up 2 days to 3 years after the injury. There were no serious complications related to the injuries. In three a friable mass of granulation tissue developed at the site of the injury; when this tissue was in the lower vagina (cases 16 and 25), a short course of topical estrogen cream allowed these wounds to epithelialize, and the symptoms resolved. A small mound of perineal granulation tissue at the wound site was cauterized when one child (case 31) was under anesthesia for colostomy closure. Three of the four patients who had been raped had chronic vaginitis when seen at follow-up; none of these three had any remaining hymenal tissue.

Six girls who were noted initially to have transected hymens, two caused by nonpenetrating objects and four among the penetrating rape cases, were seen at follow-up. Injury had healed well in all six, although the transection of the hymen remained.

Comment

Although genital injury is relatively common, when it occurs in a prepubescent girl, it is very anxiety provoking to all involved individuals. Because of its genital location and the cognitive limitations in the young child, caretakers frequently are concerned about sexual abuse. In this small series eight of 25 children (32%) were investigated for sexual abuse, even though the children gave no history nor did they have other indicators of abuse, e.g., a sexually transmitted disease or denial of injury.

Another cause for alarm is that these wounds tend to be very hemorrhagic. The external genitalia are very heavily vascularized, and a relatively minor injury can bleed excessively, particularly on the unestrogenized mucosa. The vast majority of the injuries reported here were managed conservatively in the emergency center after a history seeking the cause of the injury was obtained. However, several of the youngest and more un-

cooperative patients in this series were taken to the operative room for examination and repair of relatively minor but hemorrhagic wounds.

The anatomic feature most commonly associated with a serious wound was symmetry, which is an indication that the force of the injury was in the midline. A symmetric wound combined with hymenal transection indicates that the weakest point in the perineal floor has received the force of the causative agent. When one is dealing with injuries, other than those caused by stabs or gunshots, it is through this point in the pelvic floor that injury to internal pelvic and abdominal structures is possible.

The one group of symmetric injuries that, although painful, had minimal potential for penetration of higher viscera were caused by straddle objects; the hymen remained intact in all nine of these patients. Straddle object injuries have the potential for urethral injury and large hematoma formation, although neither of these occurred in this series.[5]

When the hymen was transected in association with a symmetric wound, not only was the potential for damage to higher viscera increased but also there was a much greater association with sexual assault. In this series deep penetrating injuries that were symmetric and transected the hymen were uniformly the result of sexual assault. It must be emphasized that the injuries caused by rape were distinctly different than the three injuries caused by nonpenetrating objects, which were also symmetric and transected the hymen. In the sexual assault survivors the vaginal introitus was clearly enlarged, and either there was a major laceration of the tissues (acute rape) (Fig. 4) or the hymen was markedly attenuated (chronic sexual abuse with acute penetration) (Fig. 1). The vaginal introitus was not widened in the acute, nonpenetrating injuries with symmetry and hymenal transections (Fig. 3).

Instrumentation of the vagina was not uniformly required and was reserved only for examinations with the patient under anesthesia. The pediatric cystoscope is an ideal instrument to examine the vagina of the prepubertal girl. Any speculum regardless of size will damage the hymen, and possibly the atrophic vaginal mucosa, and can rarely be opened sufficiently to examine the entire vagina. The small 5 mm diameter of the pediatric cystoscope will not damage the hymen, and the irrigating fluid will cleanse and expand the vaginal canal. The labia can be gently squeezed closed around the cystoscope to retain fluid and further expand the canal.

At some point attention must be given to the child's ability to urinate. Viscous lidocaine applied to the tender periurethral tissues and/or sitz baths will relieve the discomfort and spasm and allow the child to micturate. In this series no child was catheterized in the emergency center. If urethral injury is suspected, catheterization should be delayed until cystoscopy has ruled out a complete or partial urethral transection.[4, 5]

Because the location of a perineal injury does not allow it to be bandaged or splinted, allowing a child to return to normal activities too soon can be detrimental. Quite and supine activities are necessary to prevent a small hematoma from expanding and to allow approximation of tissues for secondary healing to occur. Frequent tub or sitz baths are also an important part of wound management because the area has high fecal contamination.

With the exclusion of gunshot or knife stab wounds, we believe that management guidelines can be suggested for acute genital injuries in the prepubertal girl. First, the clinician should consider what type of object allegedly caused the injury and, second, determine if the anatomic features of symmetry and hymenal transection are present.

If the object is of the penetrating type, even if the child is having minimal bleeding and/or no apparent distress, the possibility of higher visceral injury is great, especially if associated with symmetry and hymenal transection.

On the other hand, if the object was too small or too wide to penetrate higher, even if the child is screaming and has soaked several peripads with blood, she might not require a trip to the operating room, especially if the injury is asymmetric and the hymen is intact. Reassurance and/or sedation to calm the child and a pressure dressing against the wound may result in hemostasis and a more conservative approach.

We would like to thank Dr. Claudia Kozinetz for statistical analysis of the data presented.

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The use of a neural network for the ultrasonographic estimation of fetal weight in the macrosomic fetus

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The error associated with regression analysis methods for the ultrasonographic estimation of fetal weight in the suspected macrosomic fetus, approximately 10%, is clinically unacceptable. This study was undertaken to evaluate the applicability of an emerging technique, biologically simulated intelligence, to this problem. One hundred patients with suspected macrosomic fetuses underwent ultrasonographic measurements of biparietal diameter, head and abdominal circumference, femur length, abdominal subcutaneous tissue, and amniotic fluid index. The biologically simulated intelligence model included gestational age, fundal height, age, gravidity, and height. The model was then compared with results obtained from previously published formulas relying on the abdominal circumference and femur length. The biologically simulated intelligence yielded an average error of 4.7% from actual birth weight, statistically better ($p = 0.001$) than the results obtained from regression models. (Am J Obstet Gynecol 1992;166:1467-72.)

Key words: Ultrasonography, macrosomia, estimation of fetal weight, neural network, biologically simulated intelligence

Accurate estimation of fetal weight in the clinically suspected macrosomic fetus continues to be an area in evolution. Perinatal and maternal morbidity increases with larger birth weights,[1-3] making correct identification of the macrosomic infant essential. Once the fetus at risk has been identified, clinical decisions and precautions can be used to reduce morbidity.[4] Clinical estimation of fetal weight by means of the Leopold maneuvers has not been shown to be accurate.[5] Roentgenographic and computed tomographic examinations of the fetus have also not been useful, because the predictive value offers no advantage over ultrasonography and it has the concern of fetal roentgenographic exposure.[6] Ultrasonography continues to be considered the most reliable means of estimating fetal weight. Regression analysis of standard measured parameters generated in nonmacrosomic populations by Hadlock et al.[7] and Shepard et al.[8] has yielded the most common formulas for the estimation of fetal weight. Hirata et al.[9] found that Hadlock's model performs best in the macrosomic population, in spite of the fact that estimated fetal weights calculated from these formulas can vary from the actual birth weight by ≥10%. Using a study population of suspected macrosomic fetuses rather than the general population to generate regression curves has not yielded significantly better results.[10]

An emerging technique for data analysis is biologically simulated intelligence or neural network. The forte of the neural network is pattern recognition, which can be likened to regression analysis where input variables are associated with outcomes according to a specified relationship to obtain a predictive model. The actual data reduction is unique to the neural network, which is derived from models of the human pattern of thinking. The technique excels in correlating many variables that, when taken alone, may not be statistically significant but as a group provide added information to best model an outcome. This technique uses a nonlinear system of variable weighting to best model input data.

The purpose of the current study was to determine the applicability of a biologically simulated intelligence to the estimation of fetal weight in the clinically suspected macrosomic fetus. This model was then prospectively tested against the Hadlock and Shepard models.

Material and methods

The study population consisted of 102 patients seen at Los Angeles County–University of Southern California Women's Hospital, either in the clinic population

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or in the labor and delivery suite, with a clinically suspected macrosomic fetus. Excluded from the study were the fetuses of women with diabetes, anomalous infants, multiple gestations, and women whose advanced labor precluded an accurate ultrasonographic examination. All ultrasonographic measurements were conducted within 72 hours of delivery. Measurements were made with an ATL Ultramark-4 (Bothell, Wash.), a GE RT 3000 or GE RT 3600 (Milwaukee, Wis.), or a Corometrics Aloka 620 (Wallingford, Conn.). All measurements were made with a 3.5 MHz linear array transducer.

The biparietal diameter, occipitofrontal diameter, transverse abdominal diameter, anteroposterior abdominal diameter, and femur length were measured with standard techniques. Circumferences of the head and abdomen were calculated with the equation for a circle: Circumference = (Diameter 1 + Diameter 2) · 1.57. An assessment of the subcutaneous tissue was made by measuring the distance from the bony spine to the skin in the anteroposterior orientation at the level of the abdominal circumference. The amniotic fluid index was measured as previously described.[11] Calculated values were the head circumference/abdominal circumference and the femur length/abdominal circumference ratios. The other measured variable was the fundal height, which was measured in centimeters from the symphysis pubis to the fundus. Demographic variables included in the analysis included gestational age, gravidity, parity, and height of the patient.

The biologically simulated intelligence used to model the data was the Brainmaker software package (version 1.5, California Scientific Software, Sierra Madre, Calif). The input variables were gestational age, fundal height, amniotic fluid index, biparietal diameter, head circumference, abdominal circumference, subcutaneous tissue measurement, femur length, head circumference/abdominal circumference, femur length/abdominal circumference, gravidity, parity, and patient height. Data used to develop the network were taken from a previously published set of 146 patients from this institution.[7] The model was then successively iterated until the estimated fetal weight (the output variable) agreed with the actual birth weight within specified tolerance. Adjustments with respect to the number of neurons, neuron gain, and training tolerance were made to obtain maximal performance on a training subset. Prospective evaluation of the trained network was then conducted on the 102 patients described above.

Data analysis was conducted on a personal computer–based system with the CRUNCH statistical software package (Crunch Software Corp., Oakland, Calif.). The error in the estimated fetal weight is expressed as a percentage of the actual birth weight[7] as follows: Error (%) = (Estimated fetal weight − Actual birth weight)/Actual birth weight × 100. An estimated fetal weight was calculated for each patient with the neural network model, the Hadlock formula, and the Shepard formula. The Hadlock estimated fetal weight was calculated with the model: Log_{10} estimated fetal weight = 1.304 + 0.05281(abdominal circumference) + 0.1938(femur length) − 0.004(abdominal circumference)(femur length). The Shepard estimated fetal weight was calculated from: Log_{10} estimated fetal weight = −1.7492 + 0.166(biparietal diameter) + 0.046(abdominal circumference) − [2.646(abdominal circumference)(biparietal diameter)]/1000. Paired *t* tests were used to compare the difference in the mean percent error derived from the Hadlock, Shepard, and neural network models. Significance was defined as $p < 0.05$.

Results

The average birth weight in the study population was 4250 ± 350 gm, with a range of 3360 to 5260 gm. Seventy of the 101 infants (69.3%) had a birth weight ≥4000 gm, whereas 24 (23.8%) weighed ≥4500 gm at birth. The distribution of actual fetal weights when there was a clinical suspicion of macrosomia is displayed graphically in Fig. 1. A total of 62.4% (63/101) infants were ≥41 weeks' gestation. There were no anomalies or neonatal deaths in the study population.

The mean error from the actual birth weight obtained with the neural network was 4.7% ± 3.9%, whereas the Shepard and Hadlock formulas' errors were 6.5% ± 4.6% and 6.8% ± 5.0%, respectively (Table I). The neural network results were calculated with the input variables outlined in Material and Methods. Another neural network was generated with only abdominal circumference and femur length used as input variables, with a resultant mean error of 6.1% ± 4.5%.

The performance of each model over the weight range studied is shown graphically in Fig. 2. The points shown are the mean percent deviation from the actual birth weight within the weight range specified.

Comment

The ultrasonographic estimation of fetal weight in the clinically suspected macrosomic fetus is an area that continues to evolve. Previous efforts in this area have focused on correlating a few morphometric parameters, i.e., the biparietal diameter, abdominal circumference, or femur length, with birth weight. However, the error associated with these methods has been imperfect. Because most of the permutations of these morphometric measurements have been explored, it might seem reasonable to postulate that inclusion of more parameters would add to the predictive value of a model. Although a given parameter, when taken alone, does not statistically contribute to a model, an aggregate of parameters related to macrosomia may

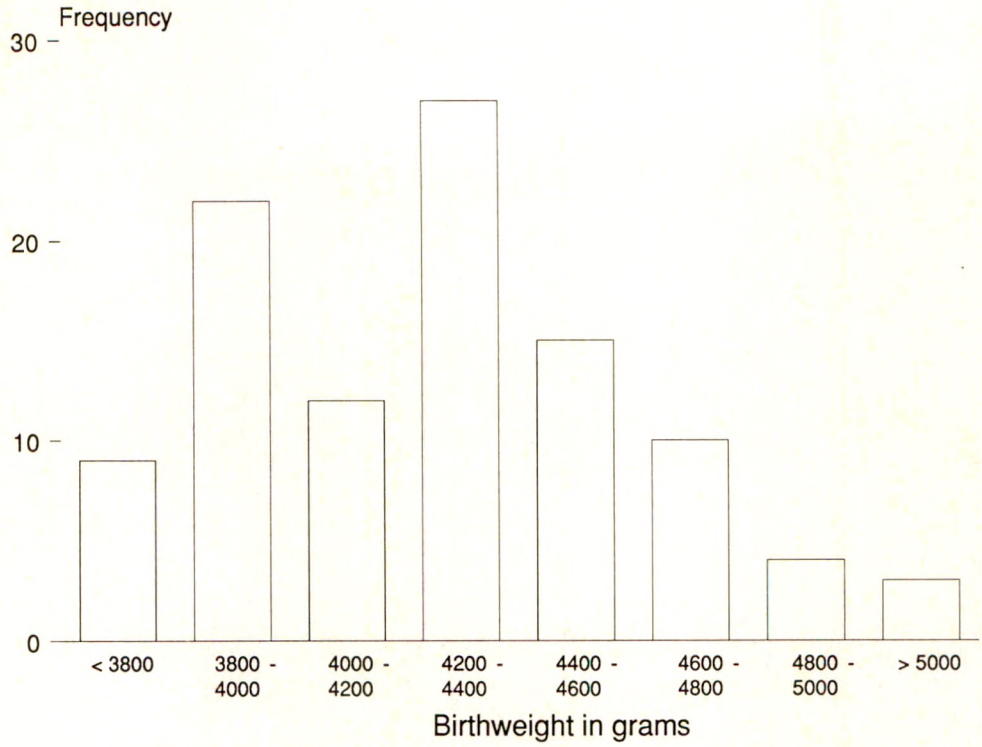


Fig. 1. Actual birth weight distribution in clinically suspected macrosomic infants.

Table I. Mean percent deviation from actual birth weight of several models for estimating fetal weight in macrosomic infants

Model	*Deviation from actual birth weight (%, mean ± SD)*	*Significance**
Hadlock	6.8 ± 5.0	$p < 0.001$
Shepard	6.5 ± 4.6	$p < 0.001$
Neural network	4.7 ± 3.9	—
Neural network (abdominal circumference and femur length only)	6.1 ± 4.5	—

*Neural network used as a comparison.

round out a model so that its performance best predicts the actual birth weight. The problem with such an approach in the past has been the unwieldy nature of the data analysis with multiple parameters.

The analysis of multiple parameters to develop a predictive model is the forte of the neural network or biologically simulated intelligence. A neural network model was first used as a research tool to model human thinking. The computer sets up a miniature model that simulates the biologic architecture of the human brain, with the terminology of the network borrowed from neurobiology (Fig. 3). The biologically simulated intelligence relies on a training data set as input, with the number of variables corresponding to the number of input neurons. The input layer is then connected to a prespecified number of neurons in the hidden layer so that all inputs are connected to all neurons in the hidden layer. Similarly, all neurons of the hidden layer are connected to an output layer, the result(s) to be modeled. The training set, matched data and outcome, is then run through the network. The network then attempts to vary the interconnections between the input-hidden layers and the hidden-output layers until the specified outcome is obtained for each input. The process is iterative, with the entire data set run through the network numerous times until all inputs match all outputs. The model is then ready for use as a predictive tool.

The neural network model is statistically more accurate than the Hadlock or Shepard formula ($p < 0.05$). The standard deviation of the percent error is minimized with the neural network model, an indi-

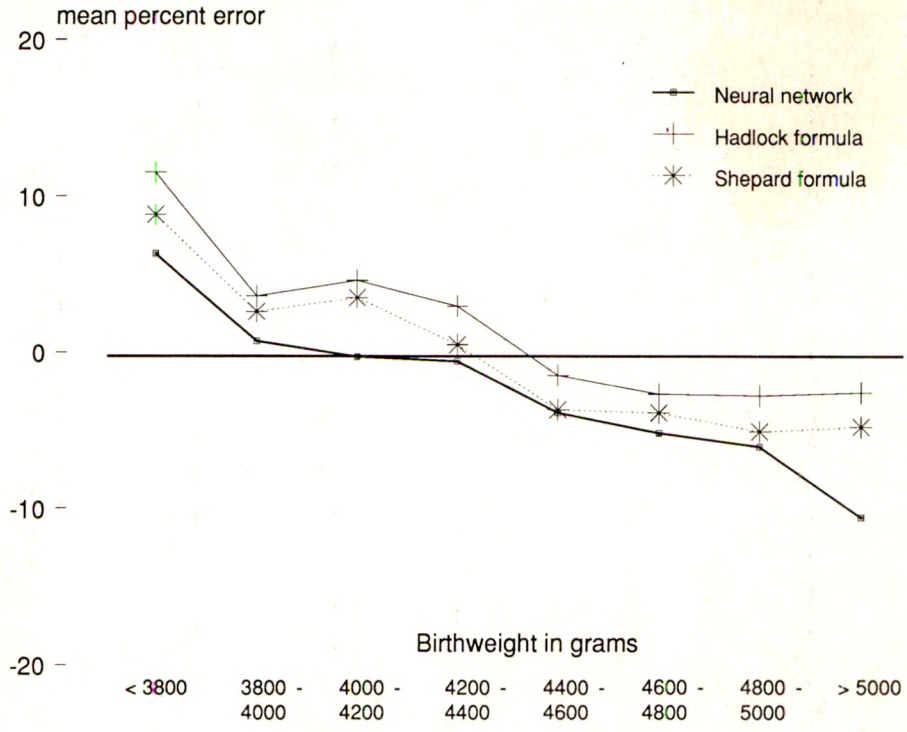


Fig. 2. Performance of various models for estimating fetal weight in clinically suspected macrosomic fetus.

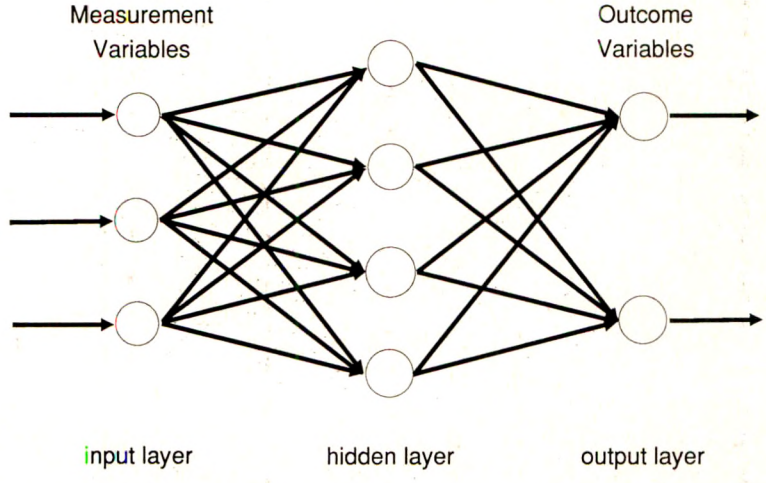


Fig. 3. Schematic representation of neural network architecture.

cation of the method's precision and accuracy. With the use of only the abdominal circumference and femur length the network model yielded results that were not statistically different from either the Hadlock and the Shepard model.

The performance of a model over the study weight range is shown in Fig. 2. It is of interest that all models have a diminished performance at the extremes of the weight range, especially when the birth weight is <3800 gm. A possible explanation for this may be operator bias on the basis of referral indication. A clinical suspicion of macrosomia existed for inclusion in the study. This clinical impression may have carried over into a bias in the measurement of morphometric parameters, particularly abdominal circumference. The result would be the systematic overestimation of fetal weight

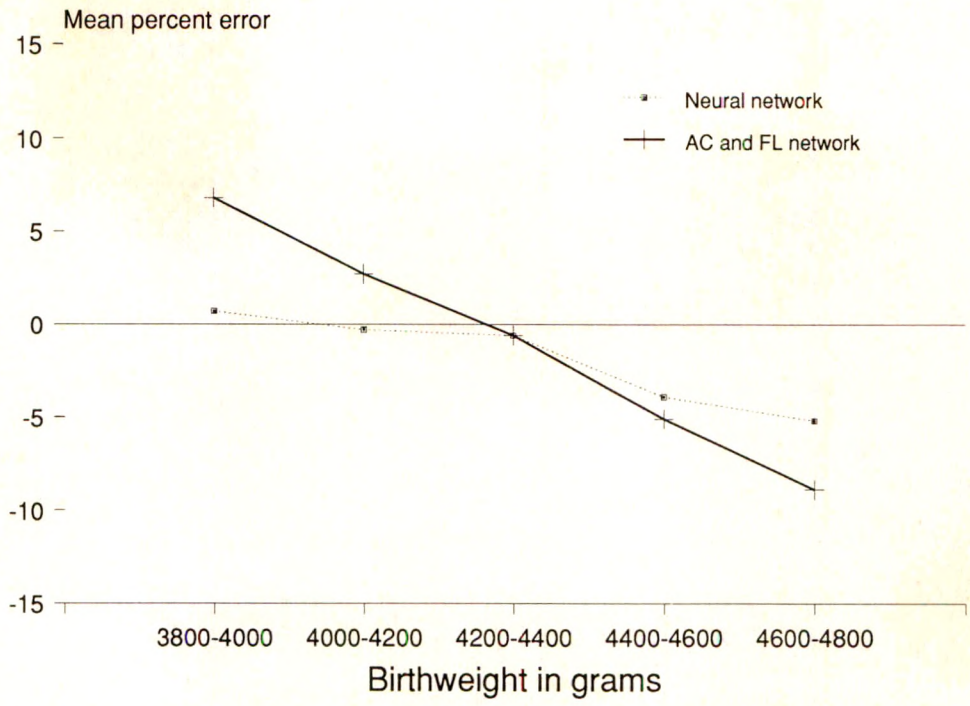


Fig. 4. Performance of two neural network models over weight range of clinical interest. *AC*, Abdominal circumference; *FL*, femur length.

Table II. Mean percent deviation from actual birth weight of several models for estimating fetal weight in the birth weight age of 3800 to 4800 gm

Model	*Deviation from actual birth weight (%, mean ± SD)*	*Significance**
Hadlock	6.5 ± 4.5	$p < 0.005$* $p < 0.005$†
Shepard	6.4 ± 4.2	$p < 0.005$* $p < 0.004$†
Neural network	4.2 ± 3.5	—
Neural network (abdominal circumference and femur length only)	4.9 ± 3.2	—

*Neural network used as a comparison.
†Neural network with only abdominal circumference and femur length used as a comparison.

when the actual birth weight was <3800 gm. All models would be expected to perform poorly because the data was biased before calculation of estimated fetal weight.

At the other end of the scale infants >4800 gm represent technical problems in the estimation of abdominal circumference because the entire abdomen may not fit on the display. This would result in estimations of abdominal circumference that would tend to favor the bias of the observer. In this study it resulted in a systematic bias toward underestimation of fetal weight in the larger weight ranges. The problems of bias exist for all the models studied. However, the underestimation of fetal weight for infants with a birth weight >4800 gm appears greater for the neural network model than for the regression models. A possible explanation for this lies in the method of data analysis particular to the neural network. The network relies on past experience with a subset of the range for accurate prediction. The model will deteriorate significantly when there are not enough data in a given area. In particular, there are only seven infants with birth weights >4800 gm. The model's predictive ability would probably be significantly enhanced by the inclusion of more infants in this weight category. Finding

such study candidates would be the major impediment to the improvement of the model in this weight range.

Exclusion of infants at the weight extremes of birth weights <3800 and >4800 gm results in the exclusion of 16 patients. The resultant mean percent error of each estimated fetal weight from the actual birth weight, with its standard deviation, is shown in Table II. The p values for the results of a paired t test comparing the neural network model with the other models are also shown in Table II. The predictive value of the neural network is significantly better than the regression models of Hadlock and Shepard. The performance of the network over the weight range from >3800 to <4800 mg is shown in Fig. 4. Inclusion of parameters other than biparietal diameter, abdominal circumference, and femur length (gestational age, fundal height, amniotic fluid index, head circumference, subcutaneous tissue measurement, head circumference/abdominal circumference, femur length/abdominal circumference, sex, age, and gravidity, parity, and height) tends to have a smoothing effect on the estimated fetal weight because the errors are less systematic than with the Hadlock or Shepard formulas.

Data analysis with the neural network is not restricted to the reduction of large numbers of variables. When only the abdominal circumference and femur length are considered, as was done in the Hadlock model, the network performed as well as the Hadlock and Shepard formulas (Table I). If the weight range is restricted to >3800 and <4800 gm (because of the previous arguments) the resultant model has a statistically greater ($p < 0.005$) predictive value than either regression formula in the macrosomic weight range. The performance of this network model, however, is not as consistent over the entire weight range as the full network model (Fig. 3).

Another interesting observation from the study is the confirmation of the inaccuracy of Leopold examinations for the estimation of fetal weight. All infants, as part of the inclusion criteria, were estimated to be >4000 gm. Only 69.3% of the infants actually had birth weights >4000 gm.

We conclude that the neural network method of data analysis is a new technique that deserves further evaluation. Its ability to integrate numerous variables to develop a predictive model offers significant potential advantages over regression analysis. Its performance in the estimation of fetal weight in the clincially suspected macrosomic fetus is better than the traditional regression analysis used by Hadlock and Shepard if multiple parameters are considered. If the parameters are restricted to the abdominal circumference and femur length, as was done with the Hadlock formula, the network still predicts the estimated fetal weight significantly better than a regression formula does. The major drawback to this form of analysis is the necessity of including a sufficient number of patients over the entire range of birth weights, with the predictive value degrading significantly when this is not done.

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Fetal echocardiography: Accuracy and limitations in a population at high and low risk for heart defects

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OBJECTIVE: Our objective was to assess the accuracy of prenatal echocardiography in detecting congenital heart defects in patients at high and low risk for structural cardiac anomalies.

STUDY DESIGN: Sixty-nine consecutive fetuses with congenital heart defects who had had prenatal ultrasonography at ≥18 weeks' gestation were evaluated to determine the accuracy of prenatal ultrasonography in identifying structural cardiac defects. Thirty-nine patients were at high risk and 30 patients were at low risk for cardiac anomalies. All fetuses were scanned with standard four-chamber and outflow tract views. Data concerning extracardiac anomalies and karyotypic abnormalities were tabulated. The accuracy of the four-chamber view alone in identifying congenital heart defects was evaluated.

RESULTS: Fifty-seven of 69 fetuses (83%) were prenatally identified ultrasonographically as having a heart defect. There was no difference in the sensitivity of detecting cardiac anomalies between high-risk and low-risk groups. When the four-chamber view was used, only 63% of fetuses were recognized as having an abnormal heart. Extracardiac anomalies were noted in 36% and karyotypic abnormalities in 17% of patients.

CONCLUSION: The four-chamber and outflow tract views done routinely in an ultrasonography laboratory seeing a mixed population of patients was successful in detecting 83% of fetuses with structural cardiac malformations. Because 43% of the fetuses with heart defects were referred for low-risk indications, systematic ultrasonographic examination of the fetal heart should not be reserved only for those at high risk. (Am J Obstet Gynecol 1992;166:1473-81.)

Key words: Fetal echocardiography, heart defects

Congenital heart defects occur in approximately eight of 1000 live births.[1] A family history of a congenital heart defect among first-degree relatives increases the risk to 4% to 12%.[2] In addition, patients with diabetes, phenylketonuria, and other noncardiac abnormalities are at increased risk for fetal cardiac anomalies. Although it is possible to catagorize certain fetuses as being at high risk for cardiac malformations, most neonates with a congenital heart defect were not perceived to be at increased risk prenatally.[3] The antenatal detection of cardiac malformations is crucial because obstetric and neonatal management is likely to be altered. The neonate with a significant cardiac lesion will benefit from delivery at a tertiary care center where the expertise of pediatric cardiologists and cardiac surgeons is readily available.

Fetal echocardiography is successful in identifying the majority of fetuses with structural cardiac abnormalities.[4-11] Most investigators have focused their attention on patients at high risk for cardiac lesions. The American Institute of Ultrasound in Medicine's guidelines advocate the use of the four-chamber view in the basic obstetric scan because this view is likely to identify disparity in chamber sizes, leading to a targeted cardiac evaluation.[12] Although some investigators suggest that the four-chamber view is adequate for detecting most cases of congenital heart disease, lesions involving the great vessels, such as tetralogy of Fallot and transposition of the great arteries, may be missed if the outflow tracts are not specifically examined.[7, 13]

Because prenatal diagnosis of congenital heart defects is critical to optimal neonatal care, we evaluated our accuracy in diagnosing congenital heart disease in a population that included patients at high and low risk for structural heart defects. In addition, we compared the sensitivity of our standard cardiac examination (four-chamber view and outflow tracts) with the sensitivity of the four-chamber view alone.

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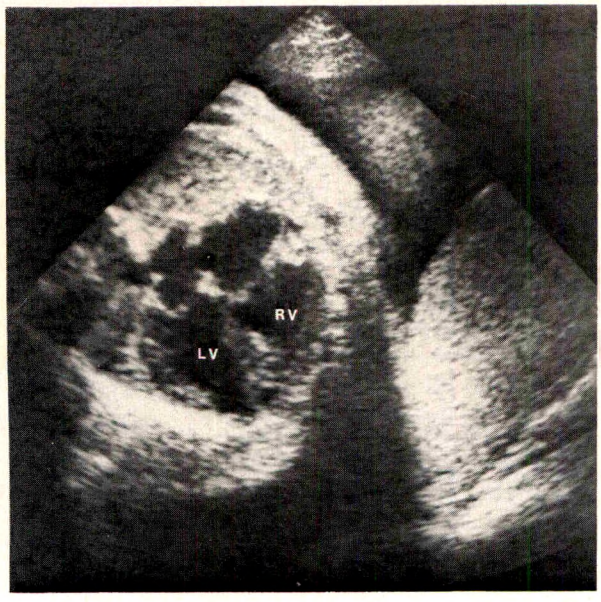


Fig. 1A. Normal four-chamber view of heart at 30 weeks' gestation. *LV*, Left ventricle; *RV*, right ventricle.

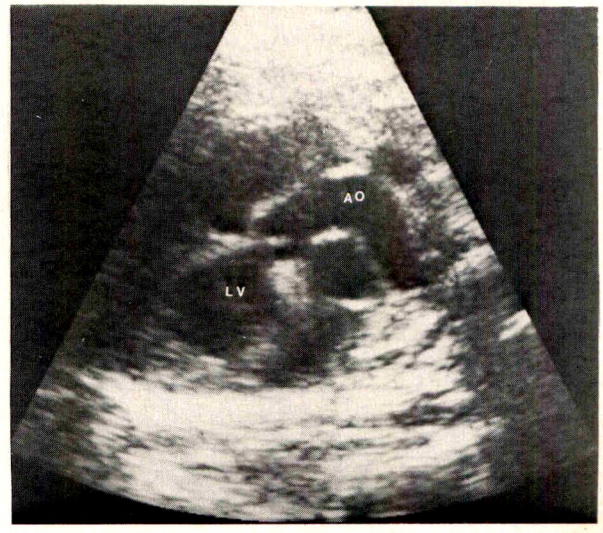


Fig. 1C. Normal long-axis view of heart showing aortic outflow tract. *AO*, Aorta: *LV*, left ventricle.

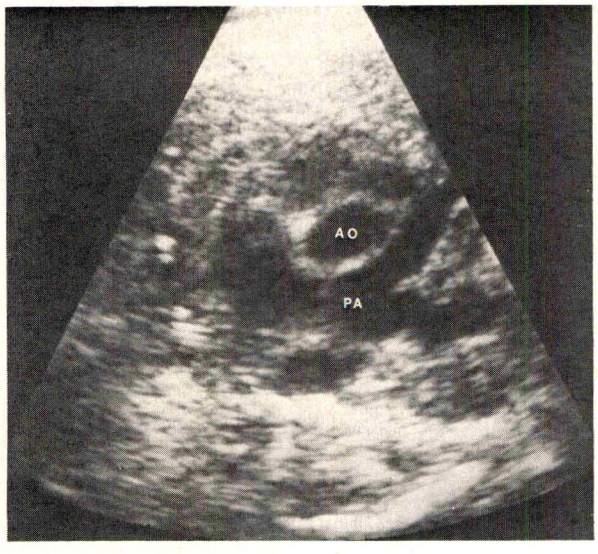


Fig. 1B. Normal pulmonary artery bifurcation. *PA*, Main pulmonary artery. Aorta *(AO)* is seen on end.

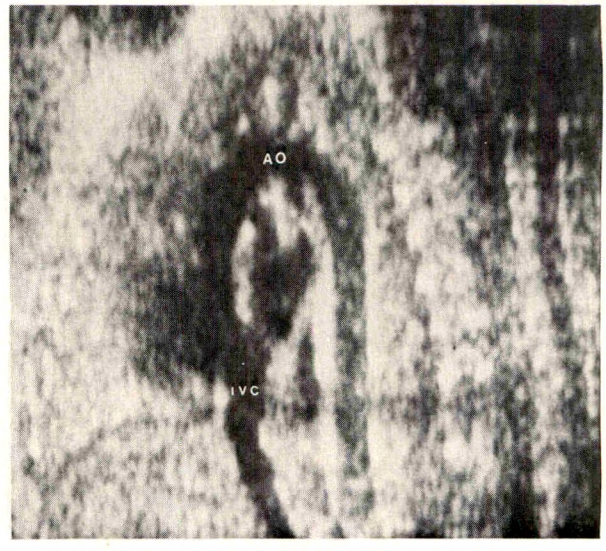


Fig. 1D. Longitudinal view of normal aortic arch *(AO)*. *IVC*, Inferior vena cava.

Material and methods

From Jan. 1, 1987, to Dec. 31, 1990, all neonates who were delivered at the Brigham and Women's Hospital or the Beth Israel Hospital and who had a diagnosis of congenital heart disease were identified with the special care nursery data base. During the same time period, all aborted fetuses delivered at the Brigham and Women's Hospital that were diagnosed as having a congenital heart defect were identified from the pathology data base. This information was then cross-referenced with records from our ultrasonography unit, yielding 69 neonates with congenital heart disease at birth and a prenatal sonogram in our ultrasonography laboratory at or after 18 weeks' gestation. The postnatal diagnosis of heart defects was made by autopsy, postnatal echocardiography, or cardiac catheterization. Neonates leaving the hospital without a recognized cardiac diagnosis were not included in the study.

Each patient in our ultrasonographic laboratory, regardless of the indication for the scan, is evaluated in a standard fashion. The cardiac portion of the examination includes the four-chamber view, short axis–long axis views, and views of the outflow tracts (Figs. 1A to 1E). Aortic and ductal arches were included when



Fig. 1E. Longitudinal view of pulmonary artery *(PA)* as it leads into ductus arteriosus *(DU)*.

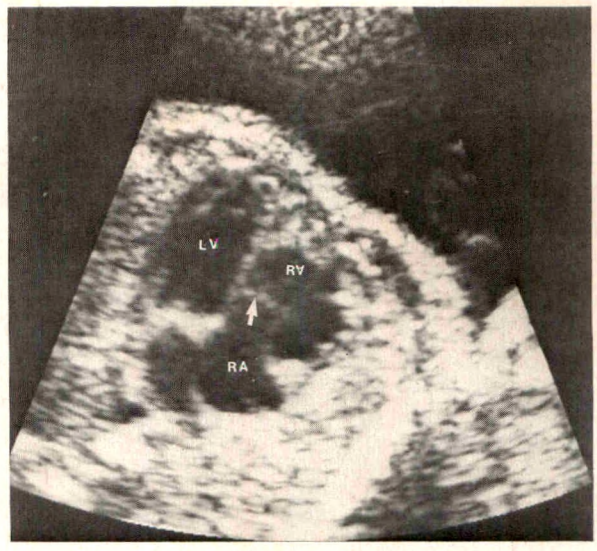


Fig. 2. Four-chamber view of heart in 25-week fetus with Ebstein's anomaly. *Arrow*, Tricuspid valve displaced in right ventricle; *LV*, left ventricle; *RV*, right ventricle; *RA*, right atrium.



Fig. 3. Longitudinal view of pulmonary artery *(PA)* in 33-week fetus with critical pulmonic stenosis. *Arrow*, Area of stenosis at pulmonic valve; *RVOT*, right ventricular outflow tract.

possible. Each scan was done with an Acuson 128 variable focus imager (Acuson, Mountain View, Calif.) with a 3.5 MHz phased array transducer.

The prenatal ultrasonographic diagnosis of congenital cardiac disease was made prospectively and taken from the original report at the time of the scan. In addition, the four-chamber views of the prenatal scans were retrospectively reviewed without knowledge of the diagnosis to determine the sensitivity of the four-chamber view alone for detecting a cardiac malformation.

The patients were divided into high- or low-risk groups according to the indication for the scan, and the accuracy for detection of heart defects was analyzed separately for each group. The indications considered to represent an increased risk for congenital heart defects includes diabetes, referral from a level I facility for suspected cardiac abnormality, referral for a suspected noncardiac abnormality, a previous child with congenital heart disease, and phenylketonuria. Patients considered at low risk were scanned for placental position, growth, uncertain dates, abnormal α-fetoprotein level, possible polyhydramnios, maternal hypertension, previous stillbirth, postdate pregnancy, twins, and preamniocentesis evaluation.

Associated anomalies and karyotypic information were also tabulated. Pediatric follow-up was obtained

Table I. Clinical diagnoses by lesion

Cardiac diagnosis	*Gestational age (wk)*	
	Correct diagnosis	*Missed diagnosis*
Hypoplastic left heart	18,18,18,19,19,20,21,24,24,27,28,34,35,36,37	20 (part of tetralogy of Fallot)
Hypoplastic right heart	18,22,25,30,33,40	NA
Endocardial cushion defect	18,18,18,18,23,24,28,28,35	18,19,24 (part of hypoplastic left heart), 36 (part of complex)
Transposition of great vessels	18,19,20,21,22,25,28,35	40 (part of hypoplastic right heart)
Tetralogy of Fallot	18,18,18,20,20,20,21,25,25,28,34	NA
Double-outlet right ventricle	18,21,22,24,24,36	17 (part of hypoplastic left heart)
Coarctation of aorta	18,19,23,28,28,35	21,22,26,30
Pulmonic atresia	28	NA
Aortic stenosis	18,26,33	NA
Pulmonic stenosis	33,34,35	24,34,34
Ventricular septal defect	18,18,18,18,19,21,23,25,27,28,31,34	18,18,18,19,20,26,26,27,27,28,34,37,40,40
Atrial septal defect	NA	18,18,18,19,19,25,28,28,34,35,37
Ebstein's anomaly	25,35	NA
Dextrocardia	18,24,36	NA
Total anomalous pulmonary venous return	NA	18,18,23,24,35,36
Interrupted inferior vena cava	25	

NA, Not applicable.

Table II. Indications for ultrasonography

Indication	*Patient*		*Lesion*	
	Correct	*Missed*	*Correct*	*Missed*
High-risk indication				
Diabetes	4	0	6	2
Cardiac referral	10	3	16	10
Family history of heart defect	7	0	10	3
Noncardiac referral	10	4	12	8
Phenylketonuria	1	0	1	1
TOTAL	32 (82%)*	7	45 (65%)†	24
Low-risk indication				
Placental location	1	0	2	1
Growth	4	0	4	1
Dating	7	1	13	4
Abnormal α-fetoprotein level	4	0	8	5
Rule out polyhydamnios	4	0	6	2
Hypertension	2	0	3	2
Previous stillbirth	1	0	1	0
Postdate pregnancy	0	2	0	2
Amniocentesis	0	2	0	2
Rule out twins	2	0	4	2
TOTAL	25 (83%)*	5	41 (66%)†	21

*By patient.
†By lesion.

for all cases of antenatally diagnosed heart defects with delivery at the reference hospital to ascertain false-positive diagnoses.

Results

Sixty-nine fetuses were delivered at Brigham and Women's Hospital or Beth Israel Hospital and found by autopsy, postnatal echocardiography, or angiography to have an identified total of 131 cardiac lesions. Fifty-seven of the 69 patients (83%) were identified prenatally as having an abnormal heart and 83 of 131 lesions (66%) were correctly detected. Table I shows the cardiac defects and gestational ages at ultrasonographic diagnosis. Nearly all cases of ventricular hypoplasia, tetralogy of Fallot, and transposition of the great arteries were correctly identified. Most lesions that were missed were part of complex cardiac defects with multiple lesions, the other parts of which were correctly identified. Other defects commonly missed included abnormalities such as atrial septal defects, ven-



Fig. 4A. Normal-appearing four-chamber view of heart in 18-week fetus with tetralogy of Fallot. *LV*, Left ventricle; *RV*, right ventricle.

tricular septal defects, and valvar stenoses. Nine of 13 endocardial cushion defects were correctly detected; of the four missed, two were part of congenital heart abnormalities identified by the other lesions. Six of 10 fetuses with coarctation of the aorta were correctly identified, usually by the disparity in size between the left and right ventricles. All cases of pulmonic atresia and aortic stenosis were detected, although only half of the cases of pulmonic stenosis were correctly seen (Figs. 2 and 3). All cases of atrial septal defects, more than half the ventricular septal defects, and all cases of total anomalous pulmonary venous return were missed.

Follow-up of all fetuses with suspected congenital heart defects on a prenatal sonogram resulted in two false-positive diagnoses. Two patients had suspected ventricular septal defects at 20 and 22 weeks' gestation, respectively, with no evidence of heart disease at birth.

Twenty-five of the 69 fetuses (36%) had other congenital abnormalities detected by ultrasonography. These abnormalities included hydronephrosis or cystic kidneys ($n = 9$), thickened nuchal fold ($n = 4$), nonimmune hydrops ($n = 3$), abnormal distal extremities ($n = 3$), hydrocephalus ($n = 3$), duodenal atresia ($n = 2$), omphalocele ($n = 2$), agenesis of the corpus callosum ($n = 2$), hemivertebra ($n = 1$), absent stomach bubble ($n = 1$), adenomatoid cystic malformation of the lung ($n = 1$), holoprosencephaly ($n = 1$), cystic hygromas ($n = 1$), and pleural effusion ($n = 1$). In addition, 12 of 69 fetuses (17%) had an abnormal karyotype, including eight with trisomy 21, three with trisomy 18, and one with trisomy 13. Two of these fetuses had no ultrasonographic abnormalities other than the congenital heart defect.

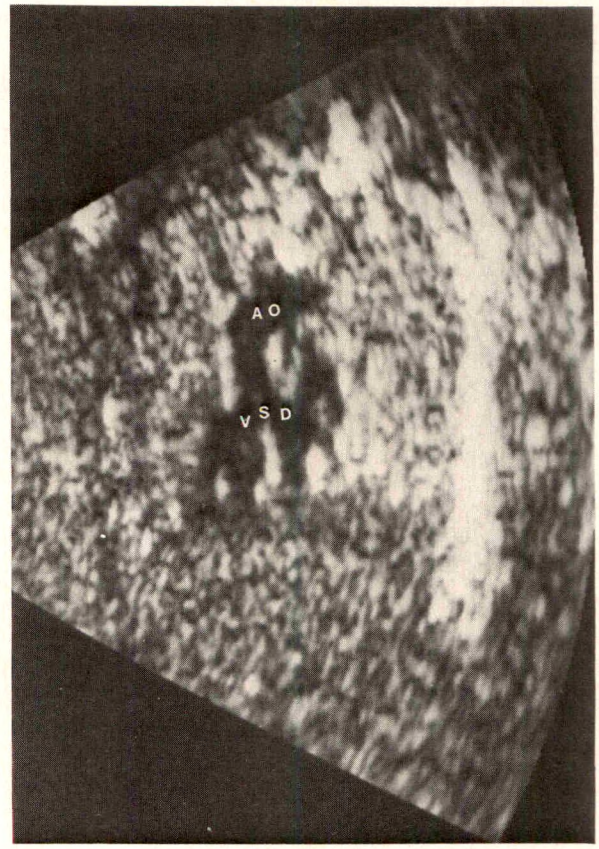


Fig. 4B. Short-axis view of same fetus showing large ventricular septal defect *(VSD)* with large overriding aorta *(AO)*.

Table II shows the various indications for ultrasonography and the correct or missed diagnoses by patient and by lesion in patients at high and low risk for congenital heart defects. Among the low-risk patients

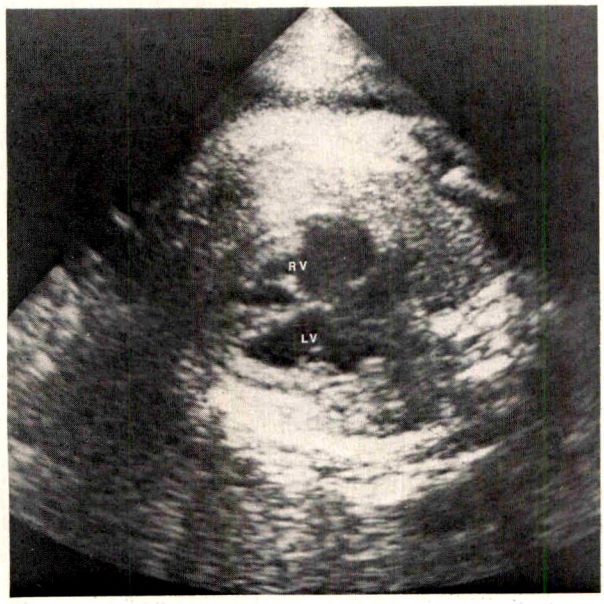


Fig. 5A. Normal-appearing four-chamber view of heart in 28-week fetus with transposition of great arteries. *LV*, Left ventricle; *RV*, right ventricle.

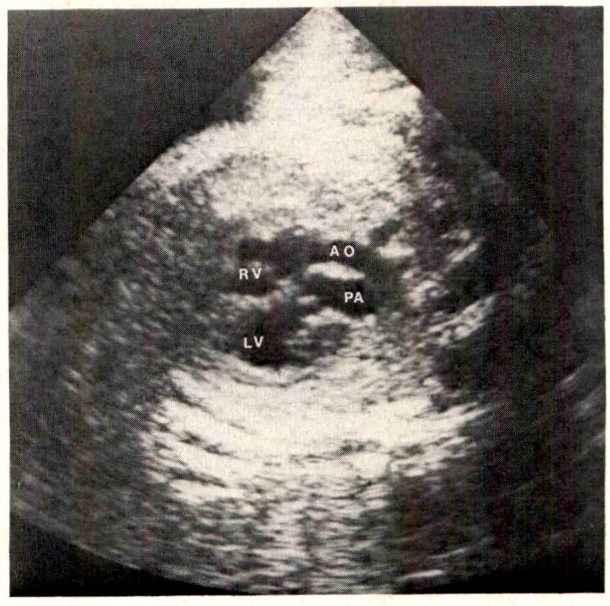


Fig. 5B. Short-axis view of same fetus showing parallel course of two great vessels. *LV*, Left ventricle; *RV*, right ventricle; *AO*, aorta; *PA*, pulmonary artery.

Table III. Comparison between standard scan and four-chamber view only

	Identified		
	No.	*%*	*Missed*
Standard scan			
Lesions	86	66	45
Patients	57	83	12
*Four-chamber view only** (n = 67)			
Patients	42	63	25

Fifteen lesions in 13 fetuses; two fetuses had two lesions missed.

*Missed on four-chamber view but identified with standard scan. Transposition of great vessels, n = 4; tetralogy of Fallot, n = 7; coarctation of aorta, n = 1; ventricular septal defect, n = 2; Interrupted inferior vena cava, n = 1.

(n = 30), 25 fetuses were correctly identified as having congenital heart defects, resulting in a sensitivity of 83%. There were 41 specific lesions correctly identified and 21 missed, for a lesion-specific sensitivity of 66%. There were 39 fetuses at risk for cardiac anomalies, 32 of whom had congenital heart defects diagnosed prenatally, resulting in a sensitivity of 82%. Forty-five of 69 lesions were correctly identified in the high-risk group, for a lesion-specific sensitivity of 65%.

On retrospective evaluation, the four-chamber view was of adequate quality in 67 of the 69 patients. The cardiac abnormality was detected in only 42 of these 67 patients (63%). Table III shows lesions with a normal-appearing four-chamber view detected prenatally with the short-axis view or views of the outflow tracts. Fifteen lesions in 13 fetuses would have been missed had the four-chamber view been used exclusively, including seven cases of tetralogy of Fallot and four cases of transposition of the great arteries (Figs. 4A, 4B, 5A, 5B, 6A, and 6B). Specifically, in the high-risk group we would have missed two fetuses with tetralogy of Fallot, two with transposition of the great arteries, and one with a ventricular septal defect. In the low-risk group analysis of the four-chamber view alone would have resulted in a missed diagnosis of tetralogy of Fallot in five fetuses, transposition of the great arteries in two fetuses, coarctation of the aorta and a ventricular septal defect in one fetus, and an interrupted vena cava in one of the fetuses with tetralogy of Fallot. Ten of the 13 fetuses with a normal-appearing four-chamber



Fig. 6A. Four-chamber view of heart in 20-week fetus with hypoplastic aorta. Note subtle discrepancy in ventricular size. *LV*, Left ventricle; *RV*, right ventricle.

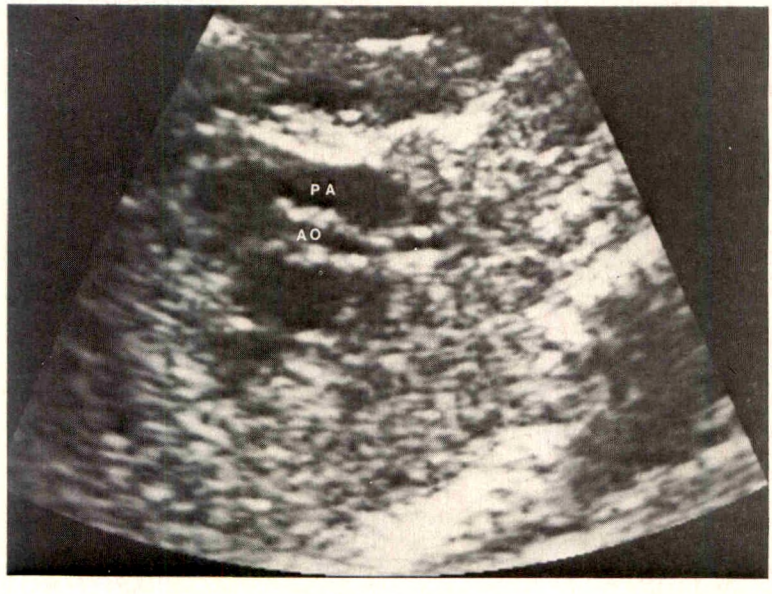


Fig. 6B. Aortic outflow tract in same fetus showing severely hypoplastic aorta, variant of hypoplastic left heart. *AO*, Aorta; *PA*, pulmonary artery.

view in spite of a heart defect had no extracardiac anomalies.

Comment

Prenatal identification of fetuses with congenital heart defects is of paramount importance, because these infants are likely to benefit from delivery at a tertiary care center where the neonate can be stabilized, the diagnosis confirmed, and corrective or palliative procedures undertaken.

Fetal echocardiography has been shown to be an accurate method for diagnosing structural cardiac malformations in fetuses at high risk.[4-11] Standard echocardiographic views permitted us to detect congenital heart disease in 57 of 69 patients (83%). Many of the lesions that were not recognized in our series were ei-

ther parts of a complex congenital heart pattern or isolated abnormalities such as septal defects, valvar stenoses, and coarctation of the aorta. These findings are in agreement with those of Crawford et al.,[8] who accurately predicted 74 cases of congenital heart disease and reported an additional 17 false-negative cases. Missed diagnoses in their series also consisted primarily of ventricular septal defects, coarctation of the aorta, and valvar stenoses. Marasini et al.[9] reported seven cases of congenital heart disease correctly identified in utero, with three false-negative diagnoses, again involving primarily ventricular septal defects and coarctation of the aorta. Parness et al.[10] correctly identified 10 of 14 cases of congenital heart disease in the midtrimester of pregnancy. Copel et al.[7] reported a high sensitivity (92%) for detecting congenital heart disease in a referral population.

Noncardiac malformations have been identified in as many as 25% to 50% of patients with congenital heart disease.[14, 15] Our data concur with previous reports; 25 of 69 fetus (36%) with heart defects had other ultrasonographically detectable anomalies. In addition, 17% of our study population had an abnormal karyotype. This is similar to the 19% incidence of aneuploidy reported by Ferrazzi et al.[11] Berg et al.[16] report the incidence of aneuploidy as 13% at birth in those patients with congenital heart disease, whereas Copel et al.[17] reported that 32% of the fetuses in their study had an abnormal karyotype. Thus we confirm the recommendation for karyotypic evaluation of the fetus with structural cardiac disease.

Although echocardiography has been advocated for fetuses at high risk for congenital heart disease, the majority of fetuses actually diagnosed with cardiac malformations have no identifiable risk factors.[3] In this study 30 of 69 fetuses (43%) identified as having a heart defect were initially referred for ultrasonography because of low-risk indications. It is our practice to examine the fetal heart in a standard fashion, regardless of the indication for scanning. In all fetuses of ≥18 weeks' gestation, we evaluate the four-chamber view and the right and left ventricular outflow tracts. Our data demonstrate that this comprehensive evaluation resulted in the identification of 83% of cardiac abnormalities in patients at low risk for congenital heart disease and that we were as successful in diagnosing congenital heart disease in the low-risk as in the high-risk populations.

Copel et al.[7] have reported a sensitivity of 92% in detecting congenital heart disease by means of the four-chamber view. In our experience evaluation of the four-chamber view alone would have allowed identification of only 42 of 67 patients with congenital heart disease, a sensitivity of only 63%. Specifically, seven cases of tetralogy of Fallot and four cases of transposition of the great arteries would have been missed. These cardiac defects can be life-threatening at birth if undetected. DeVore et al.[18] report that 7% to 10% of infants with congenital heart disease have tetralogy of Fallot and that this diagnosis is often missed on the four-chamber view. We speculate that the higher sensitivity of the four-chamber view for identifying heart defects in the study by Copel et al. is due to their patient referral pattern. Twenty of 74 patients identified were initially referred because of a suspicious level I scan (presumably an abnormal four-chamber view).

An unavoidable limitation of this study was the selection of a patient population delivered at two major teaching hospitals. It is, however, agreed that most fetuses with congenital heart defects would have been delivered at Brigham and Women's or Beth Israel Hospital because of the availability of pediatric services. Furthermore, this ultrasonography laboratory serves these two teaching hospitals primarily, and therefore the design of this study was deemed adequate.

Our sensitivity for identifying congenital heart defects has improved since our previous study published 3 years ago on a similar patient population.[3] This may be due, in part, to our ability to identify outflow tracts more consistently. It is also evident that cardiac lesions, such as atrial septal defects, ventricular septal defects, and valvar stenoses, still elude prenatal diagnosis. Further follow-up of the eight children with ventricular septal defects missed ultrasonographically (and not part of more complex cardiac defects) revealed that five did not require surgical intervention and are undergoing medical follow-up. The other three children are doing well after surgical repair. Venous abnormalities are also difficult to diagnose antenatally. We found only limited published data concerning prenatal detection of venous abnormalities, and most are associated with complex congenital heart disease.[19]

In conclusion, we believe that all obstetric scans performed at or after 18 weeks' gestation should include evaluation of the ventricular outflow tracts and a four-chamber view of the heart, regardless of whether the patient is known to be at increased risk for having a child with a congenital heart anomaly. The four-chamber view alone would not have allowed detection of 11 cases of either tetralogy of Fallot or transposition of the great arteries. Forty-three percent of fetuses with structural cardiac anomalies in this study were not considered at high risk and might have been overlooked had the fetal heart not been examined systematically. In addition, 64% of fetuses had no extracardiac abnormalities that would have led to a referral for a targeted scan. Should an abnormality in the fetal heart be detected ultrasonographically, referral to a more experienced echocardiographer is reasonable to ascertain a specific diagnosis. The patient may then be appropri-

ately counseled, and karyotypic evaluation and delivery at an appropriate institution can be undertaken.

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In situ and invasive vulvar cancer incidence trends (1973 to 1987)

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OBJECTIVE: The objective of this study was to examine recent trends in the incidence of vulvar cancer.
STUDY DESIGN: Cases of in situ and invasive squamous cell vulvar cancer were identified from nine Surveillance, Epidemiology, and End Results cancer registries.
RESULTS: The incidence rate of in situ vulvar cancer nearly doubled between 1973 to 1976 and 1985 to 1987, whereas the rate of invasive squamous cell carcinoma remained relatively stable.
CONCLUSIONS: Possible reasons for this discordance include the following: (1) Women affected by the "sexual revolution" are not yet old enough to have invasive vulvar carcinoma; (2) early diagnosis and treatment of in situ carcinoma have mitigated anticipated increases in invasive vulvar carcinoma incidence; (3) in situ and invasive carcinomas of the vulva have different etiologies, with the sexually transmitted human papillomavirus involved in the etiology of in situ carcinoma and other factors involved with most invasive squamous cell carcinoma. (AM J OBSTET GYNECOL 1992;166:1482-5.)

Key words: Vulvar cancer, incidence rates, time trends

Cancer of the vulva is rare, with an average annual age-adjusted incidence rate for invasive disease in the United States of 1.6 per 100,000 women.[1] Epidemiologic studies have shown that risk factors for vulvar cancer include multiple sexual partners, a prior infection with genital warts, current cigarette smoking, and a prior abnormal cervical Papanicolaou smear.[2, 3] Since sexual behavior and smoking practices of women in the United States have changed dramatically over the last two decades,[4, 5] an examination of recent trends in the incidence of vulvar cancer is of interest.

Material and methods

Cases of in situ and invasive squamous cell carcinoma of the vulva diagnosed between 1973 and 1987 were identified through nine population-based cancer registries participating in the Surveillance, Epidemiology, and End Results (SEER) program: Atlanta, Detroit, San Francisco–Oakland, Seattle–Puget Sound, Connecticut, Iowa, New Mexico, Utah, and Hawaii.[1, 6] Although not based on a probability sample, these geographic areas cover about 10% of the United States population. Mortality rates for the SEER program and the total United States are similar for many types of cancer and were identical for vulvar cancer in 1973 to 1977 (0.4/100,000 woman years).[1]

In this analysis we examined cancer incidence for squamous cell tumors arising in the vulva, labia, and clitoris (International Classification of Diseases for Oncology topography codes 184.1 to 184.4), including carcinomas not otherwise specified (International Classification of Diseases for Oncology morphology codes 8010, 8030, 8050 to 8081, 8123).[7] The analysis was restricted to the 2948 in situ and 2346 invasive squamous cell tumors on the basis of the assumption that the etiology of vulvar cancer, like cancer of the cervix, differs according to histologic type.[8] Non-squamous-cell malignancies were excluded, accounting for 1% and 21.8% of all reported in situ and invasive cancers, respectively. The cell-type distribution of excluded invasive cancers was as follows: basal cell carcinoma (n = 205), Paget's disease (n = 123), other and unspecified adenocarcinomas (n = 61), malignant melanoma (n = 203), sarcomas (n = 34), and other or not specified types (n = 28). Population estimates for the calculation of incidence rates were provided by SEER program staff[9] and were generally based on data from the United States Census Bureau.

Results

The annual incidence of in situ vulvar carcinoma for all races combined nearly doubled from 1.1 to 2.1 per 100,000 woman years, during the period from 1973 to 1976 and 1985 to 1987, surpassing the rate for invasive squamous cell carcinoma (Fig. 1). The largest proportional increase occurred among white women <35

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6/1/34752

years old, for whom the rate nearly tripled (Table I). Increases were more modest among black women than among white women, with the rate not quite doubling among black women <35 years old. In situ rates among blacks of all ages were higher than those among whites before 1977, but the black-white differential has diminished in more recent years. The peak in situ rate has shifted over time from women >54 years old to women aged 35 to 54.

The invasive squamous cell carcinoma incidence for all races combined was relatively stable over 1973 to 1976 and 1985 to 1987 (Fig. 1). Rates in each age group were also relatively steady, although among white women they tended to decline among those aged ≥55 (Table II). Little racial difference was evident under age 35; rates were higher at ages 35 to 54 among blacks and at ages >54 among whites. In contrast to in situ rates, invasive rates increased steadily with age. It should be noted that some of the age-specific in situ and invasive rates among blacks were based on small numbers of cases.

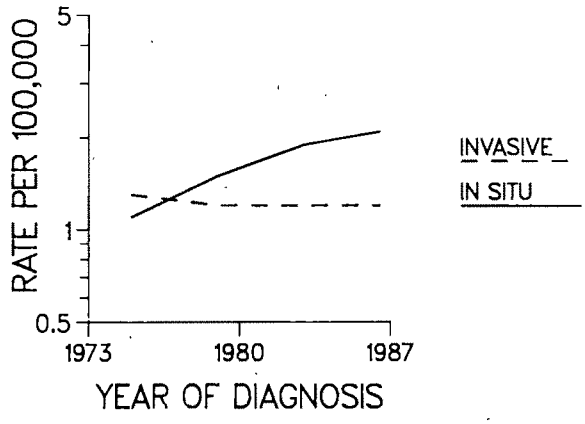


Fig. 1. Age-adjusted trends in squamous cell cancer of the vulva among all races combined (1970 vs standard).

Comment

The incidence of in situ vulvar carcinoma nearly doubled between the period from 1973 to 1976 and 1985 to 1987, with the rate among whites and younger women rising faster than the rate among blacks and older women. The increase for in situ carcinoma was not paralleled by an increase in invasive squamous cell carcinoma; its incidence was relatively steady over the same period.

We first considered whether the observed rise in the incidence of in situ carcinoma might be a data artifact resulting from changes in the classification system for vulvar intraepithelial cancer. Before 1984 lesions were divided into two broad categories: dysplasia (mild, moderate, and severe) and carcinoma in situ. Because of problems in reproducibly distinguishing severe dysplasia from carcinoma in situ, it was proposed that these two lesions be combined into one category, grade 3 vulvar intraepithelial neoplasia.[10] This revision was widely but not universally adopted by pathologists in the United States. Over the time period examined in this study, however, eight of nine SEER registries did not include cases of grade 3 vulvar intraepithelial neoplasia unless there was also specific mention of carcinoma in situ on the pathology report. Although one registry did include cases of grade 3 vulvar intraepithelial neoplasia diagnosed between 1984 and 1987, this registry contributed <5% of the total number of in situ cases reported during this time period. Our finding that the increase in the incidence of in situ carcinoma began before 1983 and continued steadily through 1985 to 1987 also argues against the revised classification scheme being responsible for the observed increase of in situ carcinoma. It is instead more likely that some in situ carcinomas have been missed and that rates of in situ carcinoma after 1983 are an underestimate of the true incidence of this condition.

The diagnosis of preinvasive disease is complicated by its similarity in clinical appearance and symptoms to some benign diseases that are commonly seen in the vulva.[11] It has also been suggested that an increasing propensity to perform a biopsy on questionable lesions might account for the observed increase of in situ vulvar cancer.[12] We have no data to examine temporal trends in the frequency of vulvar lesion biopsies. However, as the proportion of women who had ever had a Papanicolaou smear increased from 83% to 95% between 1973 and 1987,[13] opportunities for examination of the vulva in the absence of symptoms have undoubtedly increased over time. On the other hand, the greatest gains in Papanicolaou smear use have occurred among blacks and the elderly,[13] a pattern that does not readily account for the larger increases of in situ vulvar carcinoma among whites and younger women.

Although only a few epidemiologic studies of vulvar cancer have been conducted, a sexually related etiology is suspected in view of the increased risk associated with an increased number of lifetime sexual partners and a history of genital warts.[2, 3] Over the past two decades changes have occurred in the sexual practices in the United States that might explain the increased incidence of in situ vulvar carcinoma. A study conducted in 1983 revealed that the mean number of lifetime sexual partners was 1.3 and 1.9 times higher among women <35 years old than among women ≥55 years old, among blacks and whites, respectively.[4] This is consistent with the larger proportional increase of in situ vulvar carcinoma among younger women than older women and with the larger relative increase among whites compared with blacks.

In contrast, in spite of changes in sexual activity over

Table I. In situ vulvar cancer incidence by age, race, and time period in the nine SEER regions, 1973 to 1987

	<35 yr		35-54 yr		≥55 yr		All ages	
	No.	Rate*	No.	Rate	No.	Rate	No.	Rate
White women								
1973-1976	68	0.3	153	2.1	151	2.2	372	1.1
1977-1980	156	0.6	203	2.5	253	3.2	612	1.5
1981-1984	179	0.6	331	4.0	286	3.4	796	1.9
1985-1987	175	0.8	288	4.5	217	3.3	680	2.1
Black women								
1973-1976	14	0.6	22	3.1	16	3.2	52	1.6
1977-1980	24	0.7	27	3.1	9	1.4	60	1.4
1981-1984	39	1.0	35	3.5	35	5.0	109	2.3
1985-1987	29	0.9	43	5.6	14	2.4	86	2.3

*Rate per 100,000 woman years, age adjusted within strata using 1970 versus standard.

Table II. Invasive squamous vulvar cancer incidence by age, race, and time period in the nine SEER regions, 1973 to 1987

	<35 yr		35-54 yr		≥55 yr		All ages	
	No.	Rate*	No.	Rate	No.	Rate	No.	Rate
White women								
1973-1976	10	<0.1	85	1.1	411	5.4	506	1.3
1977-1980	17	0.1	90	1.2	461	5.2	568	1.3
1981-1984	17	0.1	89	1.1	500	5.1	606	1.2
1985-1987	16	0.1	65	1.1	371	4.8	452	1.2
Black women								
1973-1976	3	0.1	23	3.2	21	4.5	47	1.7
1977-1980	1	<0.1	15	1.9	28	4.4	44	1.3
1981-1984	5	0.1	13	1.4	19	2.7	37	0.9
1985-1987	3	0.1	12	1.8	20	3.4	35	1.1

*Rate per 100,000 woman years, age adjusted within strata using 1970 versus standard.

the last two decades, the incidence of invasive squamous cell carcinoma of the vulva has not increased. The apparent inconsistency between the incidence of in situ and invasive carcinoma may be because the cohort of women affected by the sexual revolution has not yet reached the age when invasive vulvar carcinoma would develop. Another possible explanation could be that early diagnosis and treatment of in situ lesions have mitigated anticipated increases in invasive carcinoma incidence. Recent data, however, support another explanation. Studies of the effects of sexual behavior on risk have focused on the human papillomaviruses as a possible etiologic agent because vulvar carcinoma is often associated with preexisting or coexisting condyloma acuminatum.[14, 15] More recently, specific human papillomavirus genotypes have been detected within invasive and in situ vulvar carcinomas.[16] Preliminary virologic evidence reveals that the majority of invasive vulvar carcinomas are not associated with human papillomavirus,[16] whereas most in situ vulvar carcinomas are human papillomavirus related,[17-19] suggesting that these two conditions may be distinct etiologic entities. Studies showing that in situ vulvar cancer rarely progresses to invasive vulvar carcinoma[15, 20] also support the view that the majority of invasive vulvar squamous cell carcinomas may be unrelated to in situ vulvar cancer, possibly explaining why changes in sexual behavior are consistent with in situ but not invasive vulvar cancer trends. Some additional support for different etiologies of these lesions is derived from the epidemiologic finding that multiple sexual partners is a stronger risk factor for in situ than for invasive vulvar cancer,[2] although this has not always been observed.[3]

Some interest has focused on the role of risk factors other than human papillomavirus in the etiology of vulvar cancer. Cigarette smoking has been shown to be a risk factor for both in situ and invasive vulvar cancer,[2, 3] with the excess risk largely restricted to current smokers. Since the prevalence of current cigarette smoking declined slightly between 1973 and 1987 among women between 20 and 64 years old and remained steady among women ≥65 years old,[4] temporal

changes in cigarette smoking are not consistent with the observed increase in the incidence of in situ vulvar cancer. Smoking also explains little of the incidence trends in invasive vulvar cancer, which were stable except for among women ≥55 years old.

Two possible but as yet unconfirmed risk factors for in situ vulvar cancer are the use of oral contraceptives and exogenous estrogens in combination with progesterone.[2,3] The cohort of women maximally exposed to oral contraceptives were in their 20s in the 1960s, 30s in the 1970s, and 40s in the 1980s.[21] Women between 35 and 54 years old in the 1980s were more likely to have been previously exposed to oral contraceptives than women in this same age group in the 1970s, consistent with the increasing trend in the incidence of in situ vulvar cancer among women between 35 and 54 years old. However, few women >55 years old during the period of this study were ever exposed to oral contraceptives. Thus oral contraceptive use most likely cannot explain the increasing trend in the incidence of in situ vulvar cancer among older women. The increasing incidence among young women also contrasts with the declining prevalence of oral contraceptive use among women <35 years old from the 1970s to the 1980s.

The prescribing of a progestin along with estrogen for menopausal women was uncommon before the mid-1980s.[22] Because its use has risen substantially since then, the future experience of older women with respect to in situ vulvar cancer should be monitored.

In summary, we have confirmed using population-based data several clinical reports that the incidence of in situ carcinoma of the vulva is increasing.[23-25] Some of the increase may be due to heightened awareness of this entity over time. However, it is more likely that changes in sexual behavior have been responsible for the observed increases. This interpretation is supported by studies that have found human papillomavirus deoxyribonucleic acid in a high proportion of vulvar carcinoma in situ lesions. Contrary to expectation, there has been no parallel increase in the incidence of invasive squamous cell carcinoma. A possible explanation for this discordance is that in situ and invasive vulvar cancer have different etiologies, as suggested by recent pathologic and virologic studies that have implicated human papillomavirus in the development of most vulvar carcinomas in situ[17] but not in most invasive carcinomas.[18-20]

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Comprehensive fetal assessment with three ultrasonographic characteristics

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OBJECTIVES: When three ultrasonographic characteristics—umbilical artery Doppler recording, growth (abdominal circumference), and biophysical profile score—are used in combination in assessment of fetuses at risk of chronic asphyxia; (1) What are the order and time scale for the development of abnormality with each characteristic? (2) What is the short-term outcome associated with abnormalities of the three characteristics? (3) Should we amend our fetal assessment protocol?
STUDY DESIGN: An audit of 103 fetuses (100 mothers) referred to a tertiary center for fetal assessment because of suspected chronic fetal asphyxia was performed with three ultrasonographic characteristics, umbilical artery Doppler recording, measurement of abdominal circumference, and documenting the biophysical profile score.
RESULTS: The order of deterioration (which had a very variable time scale) was umbilical artery Doppler recording, followed by abdominal circumference and finally biophysical profile score. Normal characteristics or an abnormal umbilical artery Doppler recording alone or an abnormal abdominal circumference alone was associated with an excellent prognosis. The worst outcome was found in the 28 fetuses with abnormality of all three ultrasonographic features before delivery.
CONCLUSIONS: The main suggested implications for management are avoidance of preterm delivery with normal ultrasonographic characteristics, an abnormal umbilical artery Doppler recording alone, or an abnormal abdominal circumference alone; delivery of fetuses at ≥34 weeks with abnormal umbilical artery Doppler recording and abdominal circumference before the biophysical profile score becomes abnormal; and implementation of specific measures to prevent necrotizing enterocolitis in newborns when all three characteristics are abnormal. (Am J Obstet Gynecol 1992;166:1486-95.)

Key words: Fetal assessment, umbilical artery Doppler, fetal growth, biophysical profile score

Ultrasonography is used to assess the fetus at risk for chronic asphyxia.[1] The three characteristics studied and used in clinical practice are umbilical artery Doppler recordings,[2] measurements of fetal size,[3] and biophysical measurements such as the nonstress test (NST)[4] or the biophysical profile score.[5] Abnormalities of umbilical artery Doppler recording and fetal growth are both associated with poor fetal outcome[6, 7]; however, they are probably affected relatively early in the pathologic process.[8] In contrast, abnormalities of biophysical measurements (NST or biophysical profile score) probably occur relatively late and reflect the degree of imminent fetal risk.[8] The clinical dilemma faced in such cases is when to deliver. Elective delivery when the umbilical artery Doppler recording or growth is abnormal may be unnecessarily premature; many of the problems of such babies after birth are related to preterm delivery rather than the asphyxial process directly.[9, 10] Conversely, leaving the fetus in utero until the biophysical profile score is abnormal may put the fetus at an increased risk.

Over the past 3 years we have followed an agreed protocol of fetal assessment in such cases, using all three ultrasonographic characteristics. The aims of this audit of our 3-year experience with this approach were: (1) to determine the order in which the three ultrasonographic characteristics become abnormal, (2) to document the short-term outcome, (3) to establish the variation in time scale of deterioration of the three characteristics, and (4) to amend, if necessary, our fetal assessment protocol.

Patients and methods

Since October 1987 patients referred to the Fetal Assessment Unit in Bristol with suspected chronic fetal asphyxia have had fetal assessment in accordance with an accepted protocol. At the first assessment structural abnormality is excluded by ultrasonography, circumferences of the fetal head and abdomen, are measured, umbilical artery Doppler recording is recorded, and biophysical profile score is obtained.[11]

Scoring for each of the five components of the biophysical profile score is in accordance with the original system used by Manning et al.,[12] namely, score of 2 each

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in a 30-minute observation for (1) one episode of fetal breathing of 30 seconds, (2) three or more discrete body or limb movements, (3) one episode of limb flexion-extension-flexion (tone), and (4) visualization of one or more amniotic fluid pockets measuring 10 mm in two perpendicular planes.

If the fetus is very small or has a structural abnormality, fetal karyotyping is also performed. If the fetus is structurally normal, the frequency of subsequent assessments depends on the umbilical artery Doppler recording and the head and abdominal circumference values. If these values are normal, umbilical artery Doppler recording and biophysical profile score are recorded weekly and growth is measured every 2 weeks. However, if the umbilical artery Doppler recording is abnormal (defined as a pulsatility index >2 SD above the mean for gestation,[13] absent end-diastolic recording, or reverse flow in diastole) or the size or growth is abnormal (defined as an abdominal circumference measurement <2 SD below the mean for gestation,[14] umbilical artery Doppler recording and biophysical profile score are recorded at least twice weekly and growth is measured every 2 weeks. The decision to deliver is taken by the consultant unit in charge of the case on the basis of both maternal and fetal condition. However, it is the policy of the Fetal Assessment Unit to advise delivery if the biophysical profile score is <8 to 10 on extended testing over 1 hour. In this 3-year audit (Oct. 1, 1987, to Sept. 30, 1990) the indications for referral and delivery were noted in each case. Perinatal outcome in terms of mortality and morbidity up to 4 weeks of age were recorded.

Statistical analysis was undertaken with nonparametric methods, χ^2 with Yates' correction for comparison of frequencies and Mann-Whitney U for comparison of medians. The level of statistical significance used in the study was $p < 0.05$.

Results

During the 3-year period 100 women had suspected chronic fetal asphyxia and were referred for fetal assessment. The indications were hypertension alone (40 patients), clinically diagnosed small for gestational age (SGA) alone (25 patients), hypertension and SGA (32 patients), and twins (three patients).

Ultrasonographic characteristics

At referral and subsequent progress. At referral the 103 fetuses could be subdivided into five subgroups according to the initial ultrasonographic characteristics.

GROUP A′: HYPERTENSION ALONE WITH NORMAL ULTRASONOGRAPHY (UMBILICAL ARTERY DOPPLER RECORDING, ABDOMINAL CIRCUMFERENCE, AND BIOPHYSICAL PROFILE SCORE). There were initially 25 fetuses in this group. The median amniotic fluid volume was 32 mm (range 23 to 59 mm). Twenty-three continued with normal ultrasonographic features until delivery. The median referral-to-delivery interval for these 23 fetuses was 1 day (range 1 to 32). Two fetuses (8%) had elevated pulsatility index values 9 and 12 days after referral, respectively.

GROUP B′: LOW ABDOMINAL CIRCUMFERENCE MEASUREMENT ONLY. All 16 fetuses in this group persisted with a low abdominal circumference as the only ultrasonographic abnormality until delivery. The median amniotic fluid volume was 29 mm (range 17 to 36 mm) and was <20 mm in only one case (17 mm). In all cases where repeated measurements were made, the abdominal circumference growth paralleled the expected normal growth trajectory but at <2 SD below the mean. The median referral-to-delivery interval for these 16 fetuses was 10 days (range 1 to 51).

GROUP C′: ABNORMAL UMBILICAL ARTERY DOPPLER RECORDING ONLY. The median amniotic fluid volume of the 20 fetuses initially in this group was 31 mm (range 29 to 40 mm). Twelve (60%) had elevated pulsatility index values as the initial Doppler abnormality, and eight (40%) had absent end-diastolic recording. In addition, two fetuses from the initial subgroup A′ hypertension alone had elevated pulsatility index values (see above); thus there were 22 fetuses with abnormal umbilical artery Doppler recording alone either at referral or subsequently. Seven of these 22 fetuses (31.8%) (all with elevated pulsatility index values and including the two from the hypertension subgroup A′) manifested no other ultrasonographic abnormality until delivery, which was 7 days later (median) (range 1 to 34 days). The remaining 15 fetuses (68.2%) subsequently manifested poor growth with the abdominal circumference measurements falling to <2 SD below the mean 21 days later (median) (range 14 to 56 days). Of these 15 fetuses with abnormal umbilical artery Doppler recording and low abdominal circumference, 13 (86.7%) manifested no other ultrasonographic abnormality until delivery. The median interval between development of a low abdominal circumference and delivery was 1 day (range 1 to 23 days). The other two fetuses (13.3%) subsequently had an abnormal biophysical profile score 3 and 7 days, respectively, from the time a low abdominal circumference was manifested. They were both delivered within 1 hour of the abnormal biophysical profile score.

GROUP D′: ABNORMAL UMBILICAL ARTERY DOPPLER RECORDING AND LOW ABDOMINAL CIRCUMFERENCE. There were initially 33 fetuses in group D′. The median amniotic fluid volume of the group was 33 mm (range 20 to 78 mm). Fourteen of these (42.4%) had elevated pulsatility index as the Doppler abnormality and 19 (57.6%) had absent end-diastolic recording. Sixteen fetuses (48.5%) (10 with elevated pulsatility index and six with absent end-diastolic recording) did not have any

Table I. General characteristics of final five fetal subgroups referred for assessment

Characteristic	Subgroup									
	A: All ultrasonography normal (hypertension alone) (n = 23)		*B: Low abdominal circumference (n = 16)*		*C: Umbilical artery Doppler recording abnormal (n = 7)*		*D: Umbilical artery Doppler recording abnormal plus low abdominal circumference (n = 29)*		*E: Umbilical artery Doppler recording abnormal plus low abdominal circumference plus biophysical profile score <8 (n = 28)*	
	No.	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
First pregnancy	18	78.3	9	56.3	5	71.4	19	65.5	22	78.6
Age										
20 yr	3	13.0	2	12.5	0		5	17.2	3	10.7
20-29 yr	13	56.5	13	81.2	5	71.4	19	65.5	15	53.6
30-39 yr	7	30.5	1	6.3	2	28.6	5	17.3	10	35.7
Indication for referral										
Hypertension*	23	100	1	6.3	4	57.1	11	37.9	1	3.6
SGA†	0		10	62.4	1	14.3	8	27.6	6	21.4
Hypertension and SGA	0		1	6.3	0		10	34.5	21	75.0
Multiple pregnancy	0		4	25.0	2	28.6	0		0	
Other problems										
Antepartum hemorrhage	2	8.7	1	6.3	0		2	6.9	1	3.6
Diabetes	2	8.7	0		1	14.3	1	3.5	1	3.6
Previous hypertension–SGA	1	4.4	4	25.0	2	28.6	4	13.8	1	3.6
Essential hypertension	2	8.7	0		0		4	13.8	2	7.1
Alcohol abuse	0		1	6.3	0		0		0	

*Sustained diastolic blood pressure ≥90 mm Hg.
†Birth weight <2 SD below mean for sex and gestation.

other ultrasonographic abnormality until delivery, which was 2 days later (median) (range 1 to 33 days). However, 17 (51.5%) (four with elevated pulsatility index and 13 with absent end-diastolic recording) subsequently manifested an abnormal biophysical profile score after a further 7 days (median) (range 1 to 26 days) and were delivered within 1 hour of the biophysical profile score.

GROUP E′: ABNORMAL UMBILICAL ARTERY DOPPLER RECORDING, LOW ABDOMINAL CIRCUMFERENCE, AND BIOPHYSICAL PROFILE SCORE <8/10. The median amniotic fluid volume in the group was 30 mm (range 12 to 90 mm), with the value being <20 mm in five of the nine cases. Seven fetuses had absent end-diastolic recording and two had reverse flow as the Doppler abnormalities. Seven of these fetuses (77.8%) were delivered within 1 hour. The eighth fetus was discovered to have trisomy 18 after a fetal karyotype determination was performed on placental biopsy material. Labor was induced, and vaginal delivery of a stillborn infant followed. In the final case, referred at 31 weeks, the fetus was considered to be too small for survival by the unit in charge of the case, and the decision was made not to perform an emergency cesarean section. Fetal death occurred within 48 hours, labor was induced, and a 750 gm fetus was delivered vaginally.

Immediately before delivery. The 103 fetuses were divided into five subgroups according to the final ultrasonographic characteristics immediately before delivery.

GROUP A: NORMAL ULTRASONOGRAPHY (UMBILICAL ARTERY DOPPLER RECORDING, ABDOMINAL CIRCUMFERENCE, AND BIOPHYSICAL PROFILE SCORE) (HYPERTENSION ALONE) (23 FETUSES). The median number of assessments performed in this group was one (range one to seven). The median amniotic fluid volume of the group before delivery was 32 mm (range 23 to 59 mm).

GROUP B: LOW ABDOMINAL CIRCUMFERENCE MEASUREMENT ONLY (16 FETUSES). The median number of assessments performed in this group was two (range one to six). The median amniotic fluid volume of the group before delivery was 29 mm (range 19 to 40 mm) with the volume being <20 mm in only one case (19 mm).

GROUP C: ABNORMAL UMBILICAL ARTERY DOPPLER RECORDING (SEVEN FETUSES). The median number of assessments performed in this group was one (range one to ten). The median amniotic fluid volume of the group before delivery was 31 mm (range 30 to 40 mm).

GROUP D: ABNORMAL UMBILICAL ARTERY DOPPLER RECORDING AND LOW ABDOMINAL CIRCUMFERENCE (29 FETUSES). The median number of assessments performed in this group was three (range one to 11). The

Table II. Obstetric characteristics of final five fetal subgroups at referral and delivery

Characteristic	Subgroup				
	A: All ultrasonography normal (hypertension alone) (n = 23)	*B: Low abdominal circumference (n = 16)*	*C: Umbilical artery Doppler recording abnormal (n = 7)*	*D: Umbilical artery Doppler recording abnormal plus low abdominal circumference (n = 29)*	*E: Umbilical artery Doppler recording abnormal plus low abdominal circumference plus biophysical profile score <8 (n = 28)*
Gestational age (wk, median and range)					
At referral	36 (28-40)	36 (31-39)	36 (28-39)	32 (26-39)	31 (25-36)*
At delivery	37 (29-40)	38 (35-41)	38 (34-40)	36 (28-40)	32.5 (26-39)†
Hypertension (No. and %)					
At referral	23 (100)	2 (12.5)	5 (71.4)	20 (69.0)	21 (75.0)
At delivery	23 (100)	3 (18.8)	6 (85.7)	22 (75.9)	22 (78.6)
Proteinuria (No. and %)					
At referral	13 (56.5)	0	1 (14.3)	9 (31.0)	16 (57.1)
At delivery	19 (82.6)	0	2 (28.6)	11 (37.9)	18 (64.3)

Hypertension is sustained diastolic blood pressure ≥90 mm Hg. Proteinuria is persistent value of 1+ or >500 mg/L.
*$A + B + C$ vs $D + E$, $p < 0.001$; D vs E, not significant.
†$A + B + C$ vs D, $p < 0.0001$; $A + B + C$ vs E, $p < 0.0001$; D vs E, $p < 0.001$.

median amniotic fluid volume of the group before delivery was 30 mm (range 11 to 75 mm) with the volume being <20 mm in five cases.

GROUP E: ABNORMAL UMBILICAL ARTERY DOPPLER RECORDING, LOW ABDOMINAL CIRCUMFERENCE, AND BIOPHYSICAL PROFILE SCORE <8/10 (28 FETUSES). The median number of assessments performed in this group was two (range one to 15). The median amniotic fluid volume of the group before delivery was 20 mm (range 5 to 90 mm) with the volume being <20 mm in 10 cases and <10 mm in only one case (5 mm).

Of the 28 fetuses with failure to achieve a normal biophysical profile score of 8/10 or 10/10, 25 (89.3%) failed to score on the NST–cardiotocograph, 23 (82.1%) on fetal breathing, 19 (67.9%) on fetal tone, 13 (46.4%) on fetal movement, and only one fetus (3.6%) had an amniotic fluid volume <10 mm.

Dexamethasone was given in six cases before delivery, three each from the final subgroups D and E.

Obstetric details. The general characteristics of the final five subgroups are summarized in Table I. When the indication for referral was hypertension alone, the likelihood of a fetus manifesting an abnormal biophysical profile score at referral or subsequently was only 2.5% (one of 40). The risks of an abnormal biophysical profile score in women referred for SGA alone or SGA and hypertension were 24% (six of 25) and 65.6% (21 of 32), respectively.

The obstetric characteristics of the final five subgroups at referral and delivery are summarized in Table II. The median gestation at referral for groups D and E (32 weeks) was significantly lower than that of groups A, B, and C (36 weeks) ($p < 0.0001$). There was no significant difference between groups D and E in gestation at referral; however, at delivery the median gestation of group D (36 weeks) was significantly greater than that of group E (32.5 weeks) ($p < 0.001$).

The delivery details of the final five subgroups are summarized in Table III. There was a high cesarean section rate for all five subgroups (the overall rate for the hospital was 10.2% in 1990). Seven of the 28 fetuses with an abnormal biophysical profile score (group E) (25%) had labor induced. In addition, four of the seven (57.1%) were delivered vaginally. Only 14 babies from group E had cord pH estimation at birth, but eight (57.1%) had a pH of ≥7.25.

Fetal karyotype. Ten fetuses (9.7%) (one in group D and nine in group E) had chromosome analysis (nine by placental biopsy and one by fetal blood sampling) after referral. One of the 10 was abnormal (trisomy 18). One baby from group E was discovered to have trisomy 18 after birth. Two more patients had karyotyping because of maternal age in the first half of pregnancy. The two cases of chromosomal abnormality were associated with large amniotic fluid volumes (75 and 90 mm, respectively).

Fetal outcome. The outcome of the five groups is summarized in Tables IV and V. All seven deaths occurred in group E. Two were fetal deaths in utero, one with trisomy 18 (31 weeks, 860 gm) and one normal fetus where the clinical decision was taken not to deliver the fetus (31 weeks, 750 gm). There were two first-week deaths. The first (29 weeks, 650 gm) was a sudden death caused by a large intraventricular hemorrhage

Table III. Delivery details of final five fetal subgroups

Characteristic	Subgroup				
	A: All ultrasonography normal (hypertension alone) (n = 23)	*B: Low abdominal circumference (n = 16)*	*C: Umbilical artery Doppler recording abnormal (n = 7)*	*D: Umbilical artery Doppler recording abnormal plus low abdominal circumference (n = 29)*	*E: Umbilical artery Doppler recording abnormal plus low abdominal circumference plus biophysical profile score <8 (n = 28)*
Indication for delivery (No. and %)					
Fetal	0	0	0	0	27 (100)
Maternal	15 (65.2)	0	1 (14.3)	10 (34.5)	0
Maturity	4 (17.4)	13 (81.2)	4 (57.1)	16 (55.2)	0
Spontaneous	4 (17.4)	3 (18.8)	2 (28.6)	3 (10.3)	0
Labor onset (No. and %)					
Induced	13 (56.5)	6 (37.5)	3 (42.8)	10 (34.5)	7 (25.0)
Spontaneous	4 (17.4)	3 (18.8)	2 (28.6)	3 (10.3)	0
Elective cesarean section	6 (26.1)	7 (43.7)	2 (28.6)	16 (55.2)	21 (75.0)
Intrapartum cardiotocogram abnormal (No. and %*)	11 (64.7)	1 (11.1)	2 (40.0)	9 (69.2)	7 (100)
Low cord pH (No.† and %)	1/2 (50.0)	0/1 (0.0)	0/0 (0.0)	1/5 (20.0)	8/14 (57.1)
Delivery by cesarean section (No. and %)	9 (39.1)	10 (62.5)	3 (42.9)	20 (69.0)	25 (89.3)

Indication for delivery: fetal, the fetal condition (biophysical profile score <8/10); maternal, the maternal condition (uncontrollable hypertension, symptoms, renal compromise, liver enzyme abnormality, or clotting disturbance); maturity, the pregnancy had reached a gestation where the risks from preterm delivery were considered small; spontaneous, spontaneous onset of labor.

*Values in parentheses represent percent of those in labor.

†Number of those tested with low values per total number tested.

with no respiratory distress on the second day of life. The second case (32 weeks, 1230 gm) was a baby with trisomy 18 who died on the second day; karyotyping was not possible because the fetus was seen on a weekend with abnormality of all three ultrasonographic characteristics. An emergency cesarean section was performed. Two babies died of necrotizing enterocolitis at 10 days (35 weeks, 1780 gm) and 16 days (29 weeks, 670 gm), respectively. The final death occurred at 10 days (27 weeks, 660 gm) from severe respiratory distress syndrome and extensive intraventricular hemorrhage.

Most of the neonatal morbidity was found in groups D and E. Furthermore, in view of the higher incidence of preterm delivery in group E, it is not surprising that babies from group E had more neonatal problems, compared with those from group D.

The differences in outcome between groups D and E could be due to the effects of preterm delivery rather than the abnormal biophysical profile score. Furthermore, nine of the fetuses in group E had an abnormal biophysical profile score and thus did not necessarily reflect any consequences of a clinical decision to wait for the biophysical profile score to become abnormal. Thus we compared the outcome of 14 normal fetuses from group E, with initially abnormal umbilical artery Doppler recording and low abdominal circumference alone who subsequently had an abnormal biophysical profile score, with those of a matched cohort of 14 fetuses from group D, who were delivered at the same gestational age but who never manifested an abnormal biophysical profile score. The outcomes are compared in Table VI. In spite of the matched gestations there were still more complications in group E. Severe complications exclusively found in this group were those that caused the two deaths, respiratory distress syndrome complicated by air leaks and bronchopulmonary dysplasia, intraventricular hemorrhage, neurologic abnormalities, and necrotizing enterocolitis. Although babies in group E had a higher incidence of respiratory distress syndrome alone and jaundice and a lower incidence of no problems, none of these differences were statistically significant.

Comment

Fetal blood gas values and acid-base status are abnormal in chronic fetal asphyxia, but recent work has suggested that measurement of these criteria obtained

Table IV. Outcome of final five fetal subgroups: Part I

Characteristic	Subgroup				
	A: All ultrasonography normal (hypertension alone) (n = 23)	*B: Low abdominal circumference (n = 16)*	*C: Umbilical artery Doppler recording abnormal (n = 7)*	*D: Umbilical artery Doppler recording abnormal plus low abdominal circumference (n = 29)*	*E: Umbilical artery Doppler recording abnormal plus low abdominal circumference plus biophysical profile score <8 (n = 28)*
Survival (No. and %)					
Survived 4 wk	23 (100)	16 (100)	7 (100)	29 (100)	21 (75.0)
Neonatal death in 2-4 wk					3 (10.8)
Neonatal death in 1 wk					2 (7.1)
Fetal death in utero					2 (7.1)
Preterm (No. and %)	8 (34.8)	5 (31.3)	3 (42.9)	20 (69.0)	25 (89.3)*
SGA	0	16 (100)	0	29 (100)	28 (100)
Apgar score <7 at 5 min (No. and %)	2 (8.7)	0	0	7 (24.1)	11 (39.3)†
Intermittent positive-pressure breathing at birth (No. and %)	3 (13.0)	1 (6.3)	0	8 (27.6)	19 (67.9)‡

SGA, <2 SD below mean birth weight for gestation and sex.
*$A + B + C$ vs D, $p < 0.01$; $A + B + C$ vs E, $p < 0.0005$; D vs E, not significant.
†$A + B + C$ vs D, $p < 0.05$; $A + B + C$ vs E, $p < 0.0005$; D vs E, not significant.
‡$A + B + C$ vs D, $p = 0.06$; $A + B + C$ vs E, $p < 0.0001$; D vs E, $p < 0.005$.

by potentially hazardous invasive procedures does not provide an accurate guide to prognosis.[9] That work demonstrated that the gestional age at delivery was the most important prognostic factor. Unanswered questions therefore remain. With chronic fetal asphyxia is it better to "buy time" by avoiding preterm delivery and allowing the opportunity for steroid therapy,[10] or is this approach associated with additional risk if the asphyxial process progresses too far in utero? Are there other ways to evaluate fetal risk? We believe the current audit starts to provide answers to these questions.

From our study we would suggest that there is a set deterioration of ultrasonographic characteristics in the fetus exposed to chronic asphyxia. First, the umbilical artery Doppler recording becomes abnormal, then fetal growth is demonstrably retarded, and finally the biophysical profile score becomes abnormal. There are two arguments to support this suggestion. First, an abnormal biophysical profile score was never encountered without an abnormal umbilical artery Doppler recording and low abdominal circumference, and an abnormal biophysical profile score and a low abdominal circumference were never found without an abnormal umbilical artery Doppler recording, yet an abnormal umbilical artery Doppler recording could be found in isolation. Second, progressive deterioration in the ultrasonographic characteristics in the suggested order was found in practice: 15 of 22 fetuses with an abnormal umbilical artery Doppler recording subsequently manifested a low abdominal circumference and two of these 15 eventually also had an abnormal biophysical profile score; 17 of 33 fetuses with an abnormal umbilical artery Doppler recording and low abdominal circumference subsequently had an abnormal biophysical profile score. The time scale of this deterioration was extremely variable, which further complicates management. In addition to the suggested order of deterioration of the three ultrasonographic characteristics, we speculate that when the biophysical profile scores becomes abnormal it also does so in a set order, with the NST and fetal breathing being early signs to be lost, then fetal movement and fetal tone.[15] Because fetal breathing is periodic,[16] the deterioration of the NST may be the more valuable early warning sign. In our experience severe oligohydramnios with an amniotic fluid volume <10 mm was a relatively rare and presumably late occurrence. This is at variance with the findings of some other workers,[15] although some of this apparent discrepancy may be explained by differences in definitions of oligohydramnios. For example, five cases in group D and 10 cases in group E had amniotic fluid pools that were between 10 to 19 mm (inclusive). However, we would contend that severe reduction in volume might be a late event because the incidence of renal failure in such babies after birth is relatively rare (Tables IV and V). Although moderate oligohydramnios (amniotic fluid volume 10 to 19 mm) reflects the severity of the intrauterine pathologic condition, we are

Table V. Outcome of final five fetal subgroups: Part II

Neonatal problem	*Subgroup*				
	A: All ultrasonography normal (hypertension alone) (n = 23)	*B: Low abdominal circumference (n = 16)*	*C: Umbilical artery Doppler recording abnormal (n = 7)*	*D: Umbilical artery Doppler recording abnormal plus low abdominal circumference (n = 29)*	*E: Umbilical artery Doppler recording abnormal plus low abdominal circumference plus biophysical profile score <8 (n = 28)*
Respiratory distress syndrome	2 (8.7)	3 (18.8)	0	5 (17.2)	8 (28.6)*
Intermittent positive-pressure breathing	2 (8.7)	0	0	3 (10.4)	8 (28.6)†
Air leaks	0	0	0	0	2 (7.1)
Patent ductus arteriosus	0	0	0	2 (6.9)	1 (3.6)
Transient tachypnea of newborn	2 (8.7)	0	0	1 (3.5)	0
Bronchopulmonary dysplasia	0	0	0	0	3 (10.7)
Polycythemia	1 (4.4)	1 (6.3)	0	4 (13.8)	4 (14.3)*
Low platelets	0	0	0	2 (6.9)	3 (10.7)*
Anemia	0	0	0	0	3 (10.7)
Sepsis	1 (4.4)	0	0	5 (17.2)	3 (10.7)†
Intraventricular hemorrhage	0	0	0	1 (3.5)	3 (10.7)
Neurologic	0	0	0	0	2 (7.1)
Necrotizing enterocolitis	0	0	0	0	3 (10.7)
Jaundice	6 (26.1)	0	0	10 (34.5)	13 (46.4)‡
Hypoglycemia	2 (8.7)	0	1 (14.3)	5 (17.2)	5 (17.9)*
Feeding problems	0	0	0	4 (13.8)	3 (10.7)
Talipes	0	1 (6.3)	0	1 (3.5)	0
Inguinal hernia	0	0	0	0	2 (7.1)
Nevus	0	0	0	0	2 (7.1)
Hypospadias	0	0	0	0	1 (3.6)
No problems	13 (56.5)	11 (68.8)	6 (85.7)	11 (37.9)	3 (10.7)§

Values are numbers and percents.

*Not significant.

†$A + B + C$ vs $D + E$, $p < 0.05$; D vs E, not significant.

‡$A + B + C$ vs $D + E$, $p < 0.005$; D vs E, not significant.

§$A + B + C$ vs D, $p < 0.05$; $A + B + C$ vs E, $p < 0.0005$; D vs E, $p < 0.05$.

of the opinion that severe oligohydramnios (amniotic fluid volume <10 mm) should remain our definition of choice for the biophysical profile score. Adopting a definition of <20 mm is likely to increase the incidence of abnormal biophysical profile score and, in turn, the incidence of preterm deliveries, with a major effect on outcome (Tables IV, V, and VI). The use of the three characteristics in combination appeared to provide a useful guide to fetal risk. If all three were normal or if an abnormal umbilical artery Doppler recording alone or a low abdominal circumference alone were the only abnormality (groups A, B, and C), the prognosis for the fetus was excellent. The majority of the mortality and morbidity was found in groups D and E, with group E fetuses tending to have the worse prognosis of the two groups. However, the question of whether this difference in outcome between groups D and E is due to a more advanced asphyxial process in utero (as indicated by the development of an abnormal biophysical profile score) in addition to the differences in preterm delivery rates is not fully resolved by this review. We would argue that there is some evidence of an effect from a more advanced asphyxial process in group E in that in the cohorts of fetuses from groups D and E matched for gestation, the severe complications (those leading to death—respiratory distress syndrome resulting in bronchopulmonary dysplasia, intraventricular hemorrhage, necrotizing enterocolitis, and neurologic abnormality) were exclusively found in group E (Table VI). Nevertheless, the incidence of other complications was not statistically different in group E. Recruitment of large numbers is required for clarification of this issue.

From our experience we would suggest modifications

Table VI. Fetal outcome of matched cohort with and without abnormal biophysical profile score

Characteristic	*Subgroup*	
	D: Umbilical artery Doppler recording abnormal plus low abdominal circumference (n = 14)	*E: Umbilical artery Doppler recording abnormal plus low abdominal circumference plus biophysical profile score <8 (n = 14)*
Gestation at delivery (median and range)	35 (27-39)	35 (27-39)
Preterm (No.)	11	11
Survived 4 wk	14 100%)	12 (85.7%)
Deaths		2*
Intermittent positive-pressure breathing at birth	4 (28.6%)	10 (71.4%)
Apgar score <7 at 5 min	2 (14.3%)	3 (21.4%)
Respiratory distress syndrome	3 (21.4%)	5 (35.7%)
Intermittent positive-pressure breathing	2 (14.3%)	2 (14.3%)
Air leaks	0	1 (7.1%)
Patent ductus arteriosus	2 (14.3%)	0
Bronchopulmonary dysplasia	0	2 (14.3%)
Polycythemia	1 (7.1%)	1 (7.1%)
Low platelets	1 (7.1%)	1 (7.1%)
Anemia	0	3 (21.4%)
Sepsis	2 (14.3%)	2 (14.3%)
Intraventricular hemorrhage	0	2 (14.3%)
Neurologic	0	2 (14.3%)
Necrotizing enterocolitis	0	2 (14.3%)
Jaundice	4 (28.6%)	9 (64.3%)
Hypoglycemia	3 (21.4%)	4 (28.6%)
Feeding problems	2 (14.3%)	2 (14.3%)
No problems	6 (42.9%)	3 (21.4%)

None of the differences is statistically significant.

*First death: 35-week, 1780 gm infant, at 10 days, of necrotizing enterocolitis. Second death: 27-week, 660 gm infant, at 10 days, of respiratory distress syndrome and intraventricular hemorrhage.

to our fetal assessment protocol for pregnancies referred to the Fetal Assessment Unit because of hypertension or SGA.

Hypertension alone and normal ultrasonographic characteristics. There is a small risk (8%) of these fetuses having a raised pulsatility index at 9 to 12 days after presentation. The severity of the maternal condition is likely to be the indication for delivery. The fetal prognosis is very good with the main risk being preterm delivery. We consider that the aims of management should be, first, to control the maternal blood pressure to prolong the pregnancy and avoid preterm delivery if possible and, second, to confirm the absence of fetal risk by serial umbilical artery Doppler recording and growth recordings only.

Low abdominal circumference only at presentation. If the abdominal circumference is low and the umbilical artery Doppler recording is normal, then it is likely that the smallness is constitutional or genetic rather than caused by chronic fetal asphyxia. The main risk to the fetus is preterm delivery. Of note, four of the 13 fetuses delivered electively in this group were, in our opinion, unnecessarily delivered preterm because of their small size alone. We consider the appropriate management to be serial monitoring of growth and umbilical artery Doppler recording. In the absence of maternal complications and with normal umbilical artery Doppler recording and growth of the abdominal circumference after the appropriate trajectory, delivery can be deferred until at least 37 weeks.

Abnormal umbilical artery Doppler recording alone at presentation. There is a 75% chance of another ultrasonographic abnormality becoming manifest 14 to 56 days (median 21 days) later. Although the umbilical artery Doppler recording remains the only ultrasonographic abnormality, the prognosis for the fetus remains very good. The aims of management are monitoring fetal growth and biophysical profile score and, if these are normal, avoiding preterm delivery.

Abnormal umbilical artery Doppler recording and low abdominal circumference at presentation. This group is the most difficult to manage. In fetuses with these two ultrasonographic abnormalities there is a 39.6% risk of the subsequent development of an abnormal biophysical profile score 1 to 26 days (median 7 days) later. Although an abnormal biophysical profile

score will therefore not develop in the majority of the fetuses, once it becomes abnormal, it may be associated with a worse outcome. Should we deliver more preterm or wait until the biophysical profile score becomes abnormal? We believe that the issue can be resolved only by a multicenter randomized trial. However, in the meantime a pragmatic approach to management is necessary. Careful ultrasonographic examination of the fetus should be carried out to exclude the stigmas of chromosome abnormality.[17] In this context it is noteworthy that both cases of chromosomal abnormality in our study had unusually large amniotic fluid volumes of 75 and 90 mm, respectively. However, even if the result of ultrasonographic examination is apparently normal, fetal karyotyping should be considered. Preeclampsia in the mother does not exclude a chromosomal anomaly. In both pregnancies with fetal trisomy 18 the mother had proteinuric hypertension. We prefer placental biopsy for karyotyping because we can obtain a result on the same day, compared with 2 days for fetal blood, during which time the fetal condition may deteriorate.[9]

Our opinion is that we should deliver the fetus that is morphologically normal with an abnormal umbilical artery Doppler recording and a low abdominal circumference at ≥34 weeks rather than wait for an abnormal biophysical profile score to develop. Perhaps this limit should be reduced to 32 weeks. Between 28 and 32 weeks steroids should be given and frequent (daily?) biophysical profile scores determined. At the first sign of biophysical profile score deterioration (probably manifest as an unreactive NST with decelerations) delivery should be undertaken. Fetal size should not be used as a major influence on this decision. Before 28 weeks we would favor biophysical profile score monitoring and the administration of steroids but would also consider delivery with persistent abnormality of the whole biophysical profile score on prolonged (60-minute) testing.

The audit did not set out to address the long-term outlook for the babies. However, development of the appropriate management strategy for fetuses with abnormal umbilical artery Doppler recording and low abdominal circumference also depends on information on the long-term neurodevelopmental progress through infancy and childhood of babies from group D compared with those from group E delivered at a similar gestational age.

Abnormalities of all three characteristics at presentation. Once an abnormal biophysical profile score has developed, there is the greatest risk of morbidity and mortality (Tables IV and V). Although this risk is largely influenced by gestation there is a suggestion of an effect from a more advanced asphyxial process in utero. With an abnormal biophysical profile score the approach to management should be positive because, in our experience, 75% of all fetuses with an abnormal biophysical profile score survived and one in 10 had no problems at all in the newborn period. Even when the biophysical profile score was abnormal at presentation, there was a 55.6% chance of survival. If structural abnormality can be excluded by ultrasonography, then delivery should be expedited. This should probably be by cesarean section because attempts at vaginal delivery are likely to be complicated by fetal distress in labor and birth asphyxia necessitating resuscitation (Table IV).

Finally, this study has implications for neonatal management. The comprehensive fetal assessment identifies a group more likely to require expert neonatal resuscitation and management of respiratory distress syndrome.[18, 19] In addition, necrotizing enterocolitis only occurred in babies delivered with a preceding abnormal umbilical artery Doppler recording, low abdominal circumference, and poor biophysical profile score. The incidence and severity of this condition may be reduced if introduction of oral food is delayed and if prophylactic antibiotics are used, although these questions also will require multicenter trials for answers.

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A model-based prediction for transvaginal ultrasonographic identification of early intrauterine pregnancy

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OBJECTIVE: Our objective was to develop a mathematic model for the prediction of transvaginal ultrasonographic identification of intrauterine gestation as a function of human chorionic gonadotropin titer or gestational age.

STUDY DESIGN: In this prospective, descriptive study normal intrauterine pregnancies of 31 patients from the Yale in vitro fertilization and embryo transfer program and infertility clinic were studied. Logistic regression analysis was used to develop a probability curve of transvaginal ultrasonographic detection of intrauterine pregnancy as a function of human chorionic gonadotropin titer or gestational age with a 5 MHz vaginal transducer.

RESULTS: A model-based prediction was constructed. It revealed that nearly all intrauterine sacs should be identified by a human chorionic gonadotropin titer of 3000 mIU/ml (first international reference preparation) or by a gestational age of 37 days on the basis of the 5 MHz vaginal transducer.

CONCLUSIONS: A model-based prediction for transvaginal ultrasonographic identification of early intrauterine pregnancy is presented that can be readily adapted by individual institutions using their own ultrasonographic equipment and human chorionic gonadotropin assay. (AM J OBSTET GYNECOL 1992;166:1495-500.)

Key words: Transvaginal ultrasonography, mathematic model, early pregnancy

The widespread availability of a radioimmunoassay for human chorionic gonadotropin furnished the clinician with the ability to make an early diagnosis of pregnancy. The development of high-resolution abdominal ultrasonography provided the clinician with the opportunity to make the early diagnosis of intrauterine gestation. The major clinical importance of this advance was in the early exclusion of ectopic pregnancy. The confirmation of an intrauterine pregnancy is considered sufficient to exclude ectopic gestation because hetertotopic pregnancy is relatively uncommon.[1-4]

The absence of an identifiable intrauterine gestational sac on abdominal and transvaginal ultrasonographic examination when the human chorionic gonadotropin titer has reached a defined threshold level has been used widely as a criteria for surgical explo-

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ration with the provisional diagnosis of ectopic pregnancy. The threshold level of human chorionic gonadotropin above which an intrauterine gestational sac is expected in a normal pregnancy by abdominal ultrasonography was initially characterized to occur at a human chorionic gonadotropin titer of ≥6500 mIU/ml, by means of the first international reference preparation.[5, 6] More recent reports have suggested an even lower threshold by abdominal ultrasonography, with intrauterine gestational sacs identified above 1800 mIU/ml, as determined by the second international standard, which was considered roughly equivalent to 3600 mIU/ml by the first international reference preparation.[7] A majority of patients with ectopic gestation, however, have titers below even the lower of these levels, and the identification of an intrauterine gestation would thus not be expected.[7] The ability to make an earlier diagnosis of ectopic pregnancy would therefore be enhanced if the threshold level for reliable detection of an intrauterine gestation were lower.

The transvaginal ultrasonographic approach has been used extensively for follicular monitoring during ovulation induction in programs of in vitro fertilization where it has been found to provide both increased visualization of the pelvis and better resolution than the transabdominal approach.[8] Furthermore, recent data have demonstrated the enhanced ability of the transvaginal approach in visualizing the gestational sac of early pregnancy whether intrauterine or extrauterine at an even earlier gestational age than reported with the transabdominal approach.[9-19] Initially these latter studies focused on the earliest possible detection of an intrauterine gestational sac and relied on several estimations to determine the point at which an intrauterine gestational sac should be expected. The ability to determine the maximal human chorionic gonadotropin titer or day of gestation when an intrauterine gestation should be reliably detected is the parameter of most clinical importance.

The purpose of the current study was to determine the human chorionic gonadotropin titer and gestational age at which the appearance of an intrauterine gestational sac can be expected to be identified by transvaginal untrasonographic examination in a viable, singleton intrauterine pregnancy on the basis of a 5 MHz transvaginal probe. A method is described that can be adapted by individual institutions to determine the probability of sac identification at a specific human chorionic gonadotropin titer or gestational age with the respective institution's ultrasonographic equipment and human chorionic gonadotropin assay.

Material and methods

Patients in the Yale in vitro fertilization and embryo transfer program and other patients in the infertility clinic were enrolled in the study immediately on confirmation of pregnancy by positive serum human chorionic gonadotropin titers. Patients who subsequently were verified to have normal, singleton intrauterine gestations were used for the calculations. There were eight patients from the in vitro fertilization and embryo transfer program, 17 patients who underwent ovulation induction with menotropins, and six patients with spontaneous pregnancies. Serum human chorionic gonadotropin titers were measured in samples drawn on the day of ultrasonographic examination; they were determined by an immunoradiometric assay and a double-antibody technique with the first international reference preparation as a reference standard, the details of which have been previously described.[5, 6, 9] The day of gestation was calculated from the day of human chorionic gonadotropin injection. Gestational age data were used only from in vitro fertilization patients and those undergoing ovulation induction who received human chorionic gonadotropin for initiation of ovulation.

The patients were then examined with transvaginal ultrasonography usually at 1- to 4-day intervals as determined by patient availability. The ultrasonographic examinations were performed using a model RT 3000 General Electric ultrasound machine (Rancho Cordova, Calif.) and a 5 MHz vaginal transducer. All examinations were performed in real time in a routine, systematic manner with each study including both transverse and longitudinal scans. All examinations and interpretations were completed by a single observer (B.S.S.) independent of the patient's human chorionic gonadotropin titer at the time of each examination. Thus patients were monitored with serial ultrasonographic examinations to determine the highest titer or gestational age at which an intrauterine sac was not identified and then the point at which it was subsequently identified by ultrasonography.

Statistical analysis to calculate the probability of gestational sac appearance as a function of human chorionic gonadotropin titer was performed by both nonparametric and parametric methods with an interval censoring technique[20, 21] and logistic regression analysis, respectively. A probability curve for the detection of an intrauterine sac at each human chorionic gonadotropin titer and gestational age was then constructed from the logistic regression curve.

Results

In this study group the lowest titer of human chorionic gonadotropin at which an amniotic sac was identified occurred at a level of 912 mIU/ml (first international reference preparation). The highest titer at which a sac was not detected but was subsequently identified occurred at a titer of 2550 mIU/ml (first international reference preparation). There was an overlap

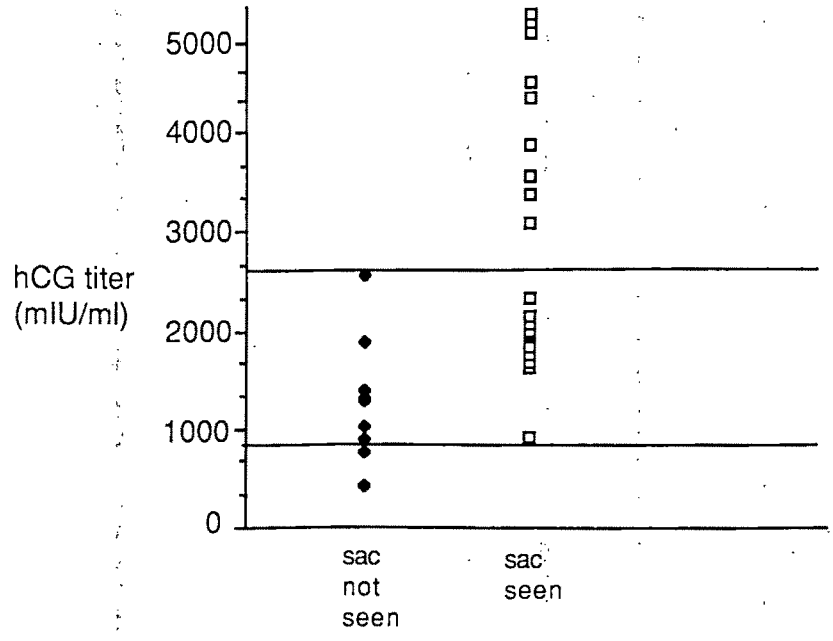


Fig. 1. Human chorionic gonadotropin titers at which sacs may or may not be identified from 912 to 2550 mIU/ml.

of human chorionic gonadotropin titers at which sacs may or may not have been identified between 912 and 2550 mIU/ml (first international reference preparation) (Fig. 1). When determined by gestational age, the highest titer at which a sac was not detected but subsequently identified occurred on cycle day 34, with the earliest detection of a sac occurring on day 31. Between these two points there was also an overlap of gestational ages during which time an intrauterine pregnancy may or may not have been identified.

A probability curve for intrauterine gestational sac identification was constructed with the logistic regression model as a function of both human chorionic gonadotropin titer (Fig. 2) and gestational age (Fig. 3). The step function corresponds to the observed probability of sac identification derived from the data, whereas the smoothed curve represents the model-based probability of sac identification, which is described by the function $y = 1/1 + \exp [\beta 0 + \beta 1 \ln (\text{human chorionic gonadotropin})]$, where y = probability of appearance as a function of the human chorionic gonadotropin titer, $\beta 0 = 41.78$, and $\beta 1 = -5.64$. With x^2 used as a measure for the fitness of the curve, $\chi^2 = 12.55$ and $p < 0.001$. Similarly, the model-based probability for sac identification as a function of gestational age is described by the function $y = 1/1 + \exp [\beta 0 + \beta 1 (\text{days})]$, where y = probability of appearance as a function of the gestational age in days, $\beta 0 = 12.35$ and $\beta 1 = -0.60$. With χ^2 used for measuring the fitness of the curve, $\chi^2 = 8.1$ and $p = 0.004$. From these curves it can be seen that 50% of the gestational sacs could be identified by a human chorionic gonadotropin titer of 1500 mIU/ml (first international reference preparation) or at a gestational age of 32 to 33 days in a normalized 28-day cycle. Furthermore, from the logistic regression model it can be seen that nearly all intrauterine sacs should be identified by a human chorionic gonadotropin titer of 3000 mIU/ml (first international reference preparation) or by a gestational age of 37 days in a singleton intrauterine pregnancy when the 5 MHz vaginal transducer described is used.

Although the highest titer without identification of an intrauterine gestation occurred at a human chorionic gonadotropin titer of 2550 mIU/ml (first international reference preparation) and by a gestational age of 34 days, the model predicts somewhat higher values at which nearly all of the sacs should be identified. This is explained by the fact that the model takes into account the inherent variability of future measurements.

Comment

The concept of a discriminatory zone was originally established to determine human chorionic gonadotropin titers at which an intrauterine sac should be seen.[5] Early reports have revealed earlier detection in the evaluation of intrauterine pregnancy with the transvaginal approach than those reported with transabdominal ultrasonograhpy.[10, 11] These studies reported ranges of human chorionic gonadotropin titers at which intrauterine pregnancies could be identified, but, because

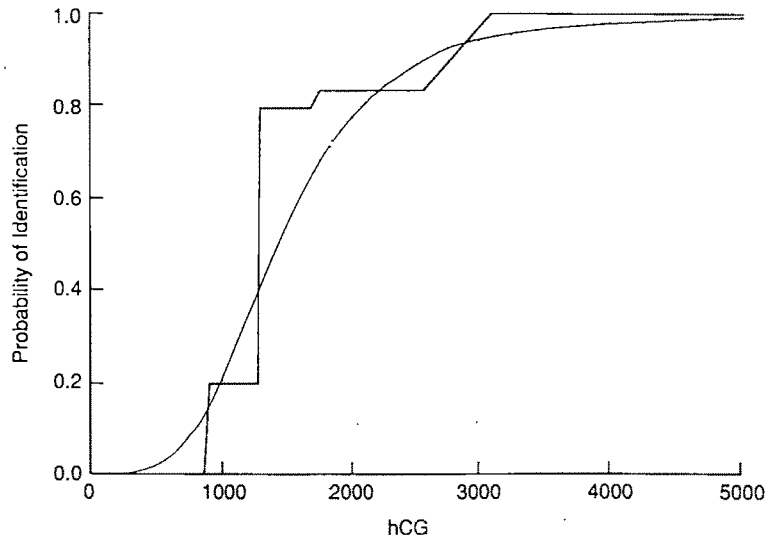


Fig. 2. Model-based probability curve for intrauterine gestational sac identification by human chorionic gonadotropin titer.

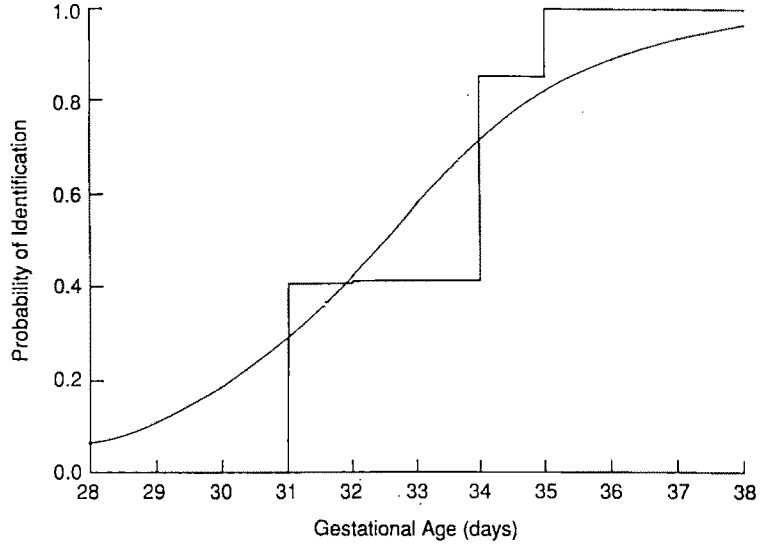


Fig. 3. Model-based probability curve for intrauterine gestational sac identification by gestational age normalized to a 28-day cycle.

the major importance of ultrasonographic identification is in knowing when an intrauterine gestational sac should be identified, reporting the range of human chorionic gonadotropin titers at which intrauterine pregnancies could be identified is of little direct help to the clinician. Furthermore, reporting only the range together with the minimum and maximum values at which intrauterine sacs are identified can be misleading because of an inaccurate representation of the true statistical distribution at which points identification may or may not occur. The latter is true because ultrasonographic identification of the intrauterine gestational sac represents a skewed, long-tailed statistical distribution. At the extreme end of the distribution, represented by either the titers or gestational age, the highest titers or oldest gestational ages at which sacs cannot be identified become progressively smaller, whereas the number of sacs identified in this range becomes progressively larger. The use of minimum and maximum values as summary statistics fails to provide information about the probability of appearance within a given range of either titer or gestational age of sac identifi-

cation and thus overlooks the most important facet of this type of study, which is to determine the probability of sac identification at any specific titer or gestational age. Futhermore, when the sample size is neglected in the determination of expected sac identification, an inaccurate representation of the points at which sacs are identified occurs at the extreme end of the distribution curve for identification, giving the appearance of an exact point above which all sacs should be identified. With the use of a mathematic model and interval censoring techniques, as well as the incorporation of sample size into the calculation, such as in a logistic regression model, the specific likelihood of identification can be calculated as a function of either human chorionic gonadotropin titer or gestational age. The clinician can therefore know the likelihood of sac identification at a particular human chorionic gonadotropin titer or gestational age. Thus, for example, at a human chorionic gonadotropin titer of 1500 mIU/ml (first international reference preparation) or between 32 and 33 days' gestation, 50% of the sacs should be visualized and 50% will not be visualized in a normal singleton intrauterine pregnancy in the current study.

It should be noted that the maximum values observed in a sample of the population are always going to be less than or equal to the actual maximum values, and conversely the minimum values observed are always greater than or equal to the actual minimum values in the true population. Thus, as more data are accumulated with the same transvaginal probe, the detection of an intrauterine gestational sac will be reported at successively lower titers and earlier gestational ages, whereas the highest titer and gestational age at which all sacs are identified will become progressively greater. The latter result will tend to be mitigated, however, with the use of transvaginal probes having greater resolution, so that the size and the gestational age at which most of the sacs are identified may be lower overall. Recent studies have attempted to describe mathematically the correlation of human chorionic gonadotropin titer and sac size with simple regression analysis correlating sac size with human chorionic gonadotropin levels to determine a threshold point of human chorionic gonadotropin titer at which a sac should be seen.[12, 14, 18] However, these studies have failed to address the limitations imposed by extrapolating to the general population threshold values determined from the study of a specific sample size. In the current study the authors have constructed a model-based prediction with logistic regression analysis to predict the probability of seeing a sac at a selected value of human chorionic gonadotropin or a selected gestational age that incorporates the sample size into the calculation.

Athough the identification of the gestational sac may be expected at the limits of resolution of the equipment, this merely represents the theoretic limits of detection. Biologic variability in singleton gestations and the possibility of inflated serum human chorionic gonadotropin values from multiple gestations must be considered in the routine clinical use of these data. Furthermore, other confounders are possible when such small sacs are viewed. The presence of small amounts of fluid such as blood can easily be mistaken for a sac in the presence of decidualized endometrium. Thus the presence of the pseudosac in ectopic pregnancy may also be difficult to distinguish from an early pregnancy. For these reasons, although the identification of an intrauterine sac is reassuring, it is important to correlate the findings with serial human chorionic gonadotropin titers and serial examinations.

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Influence of labor and oxytocin on in vitro leukotriene release by human fetal membranes and uterine decidua at term gestation

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OBJECTIVE: The purpose of this study was to determine whether labor and oxytocin influence in vitro leukotriene release by human fetal membranes and uterine decidua at term gestation.
STUDY DESIGN: All women ($N = 27$) were studied in an academic clinical research environment. They were delivered either vaginally or before labor by cesarean section. Tissues were incubated with calcium ionophore A23187 alone or with calcium ionophore A23187 after preincubation with either oxytocin, BW755c, or cycloheximide. Leukotrienes in culture medium were determined by high-performance liquid chromatography. Statistical analysis was performed with analysis of variance.
RESULTS: Tissues obtained after vaginal delivery released significantly higher ($p < 0.05$) quantities of leukotrienes LTB_4, LTD_4, and Δ-6-trans-LTB_4 than did tissues obtained during cesarean section. Leukotriene release was stimulated by oxytocin and was inhibited by both BW755c and cycloheximide.
CONCLUSION: These results suggest an activation of the arachidonate lipoxygenase pathway in these tissues during labor. Oxytocin could play a regulatory role in this process. (Am J Obstet Gynecol 1992;166:1500-6.)

Key words: Leukotrienes, human fetal membranes, oxytocin, parturition

The role of cyclooxygenase derivatives of arachidonic acid in the biochemical events leading to human labor has been extensively investigated.[1] There is a consensus that prostaglandins PGE_2 and $PGF_{2\alpha}$ are involved both in myometrial contractions and in the ripening of the uterine cervix.[2, 3] The hormonal factor(s) regulating prostaglandin production in labor have not been fully elucidated.

In contrast, little is known about the lipoxygenase derivatives of arachidonic acid in labor. The leukotrienes have potent biologic actions in nonreproductive tissues.[4] Recent studies suggest that leukotrienes could play a role in the biochemical processes of labor in the human[5-7] and in the nonhuman primate.[8]

This study was undertaken to examine the in vitro release of leukotrienes by human fetal membranes and uterine decidua, to verify whether differences in the production of these substances could be detected in relation to the presence or absence of labor, and to investigate whether oxytocin could stimulate leuko-

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triene synthesis and release by these tissues. This role for oxytocin could be suggested because it has been previously shown that oxytocin induces an increased release of cyclooxygenase arachidonate metabolites by human decidua at term gestation.[9]

Material and methods

Subjects. Twenty-one healthy women (age range 24 to 32 years) were included in the study. These patients had singleton uncomplicated pregnancies at term. They were divided into three groups: Group 1 included seven women who were delivered vaginally after spontaneous labor without medication until after delivery; groups 2 and 3 each included seven women who were delivered by elective repeat cesarean section before labor. All patients who underwent cesarean section received a general anesthetic (atropine sulfate 0.02 mg/kg, thiopental sodium 4 mg/kg, pancuronium bromide 0.08 mg/kg, nitric oxide 60% plus oxygen 40%) until the fetus was delivered.

Gestational age, which was calculated by the date of the last menstrual period and was confirmed by ultrasonographic examination, was comparable in the three groups. Birth weights and Apgar scores were also comparable.

Sample collection and tissue culture. Reflected fetal membranes with adherent decidua were obtained after spontaneous vaginal delivery (group 1) and during repeat cesarean section before labor (groups 2 and 3).

Specimens were placed in ice-cold sterile saline solution and were immediately sent to the laboratory for processing. All specimens were processed as soon as they reached the laboratory.

Tissues were layered on Petri dishes supported by ice and were washed several times with ice-cold sterile culture medium M199 (Gibco, Grand Island, N.Y.) to remove blood. Clots were carefully and gently removed with fine forceps, and additional washing completely removed blood. Tissues were cut with fine scissors into disks. The areas in which the amnion was partially detached from the chorion were discarded. Tissues were quantified by wet weight, were placed in Petri dishes containing 5 ml of culture medium M199 and were incubated at 37° C in an atmosphere of 95% oxygen and 5% carbon dioxide.

Samples obtained from subjects of groups 1 and 2 were divided into three specimens. They were incubated in the presence of the calcium ionophore A23187 (Sigma Chemical Co., St. Louis), 10 μmol/L. Previous time course analysis, which had been carried out at 30, 60, and 90 minutes of incubation, had demonstrated maximum leukotriene release after 60 minutes of incubation with ionophore A23187. For this reason all subsequent experiments were carried out at 60 minutes of incubation.

The samples were preincubated for 30 minutes with 40 mIU oxytocin (Syntocinon; Sandoz, Basel). At the end of the preincubation period, tissues were washed twice with fresh medium and then incubated for an additional 60-minute period in the presence of the calcium ionophore A23187, 10 μmol/L. Control samples were incubated without calcium ionophore.

Tissues obtained from the subjects of group 3 were preincubated 30 minutes at 37° C with oxytocin at four different concentrations (10, 20, 40, and 60 mIU/ml) and treated as described previously. Tissue viability, judged by the lactic dehydrogenase assay,[10] was 89% ± 4% before incubation and 80% ± 3% at the end of the incubation. No difference was found in tissue viability between samples obtained after cesarean section before labor and those obtained after spontaneous delivery.

To verify whether leukotriene release into the culture medium was due to active secretion and not to our experimental manipulations, we performed additional experiments in which some specimens from group 1 were coincubated either with 0.5 μg/ml cycloheximide (Sigma) or with BW755c (Wellcome Research Laboratories), 100 μmol/L, for 15 minutes and then challenged with 10 μmol/L calcium ionophore. Culture medium from every experiment was collected, centrifuged at 1800 rpm for 15 minutes to eliminate cell debris, and stored at −80° C until the successive assay was undertaken.

Analysis of leukotrienes. We used Powell's modified method, described in detail elsewhere,[7] for leukotriene extraction.

Dried fractions obtained after extraction were resuspended in 100 μl methanol and filtered. The clear solution was applied to an Ultrasphere C18 column (5 μm particle size; Beckman Instruments, Inc., Bioanalytical Systems Group, San Ramon, Calif.) with methanol/water/acetic acid (73:27:0.1), pH adjusted to 4.7 with ammonium hydroxide, used as the mobile phase. A flow rate of 1 ml/min was maintained, and the effluent was monitored at 280 nm with an LC 95 Perkin Elmer Detector and a Perkin Elmer 235 Diode Array Detector to obtain ultraviolet spectra, absorbance maxima, and purity indexes. Retention times were calculated by coelution with authentic standards. The peak areas or heights were calculated with an LCI 100 Laboratory Computing Integrator (Perkin Elmer). Standard curves of the individual leukotrienes were obtained with five different concentrations (1.5625 to 25 ng) and showed the following correlations: For LTB_4, $r = 0.978$ and $y = 56.224 \pm 53.59x$; for LTD_4, $r = 0.945$ and $y = 18.54 \pm 13.28x$; for Δ-6-trans-LTB_4, $r = 0.983$ and $y = 116.8 \pm 51.28x$. The minimum detectable quantities were 1.5 ng for LTD_4 and 1.0 ng for LTB_4 and for Δ-6-trans-LTB_4.

Leukotriene recovery was calculated by adding a known amount to 1 ml of culture medium and pro-

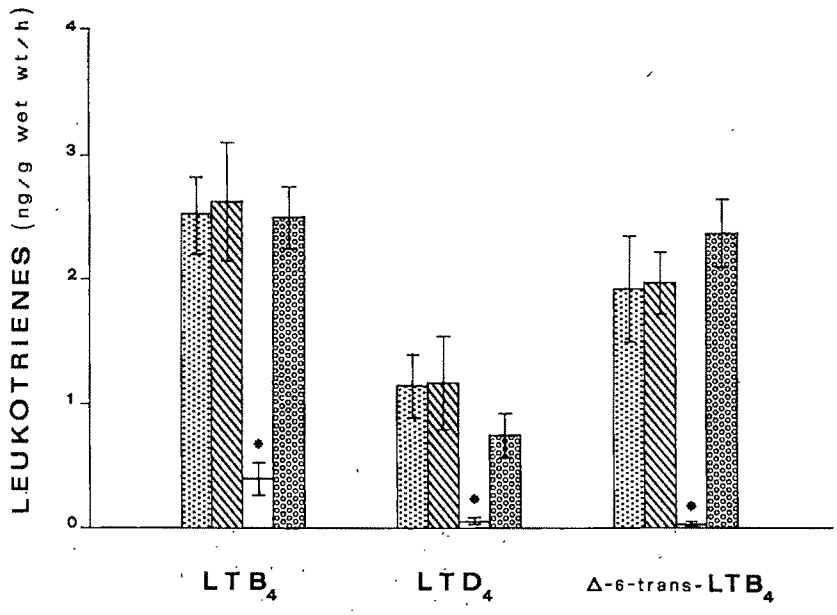


Fig. 1. Leukotriene release by human fetal membranes and uterine decidua at term gestation. Group 1: Tissues were obtained after spontaneous delivery. They were either incubated with calcium ionophore A23187 10 μmol/L for 60 minutes (▨, $n = 7$) or preincubated for 30 minutes with oxytocin 40 mIU/ml, washed, and then incubated for 60 minutes with calcium ionophore A23187, 10 μmol/L (▧, $n = 7$). Group 2: Tissues were obtained during elective cesarean section before labor. They were either incubated with calcium ionophore A23187, 10 μmol/L for 60 minutes (□, $n = 7$) or preincubated for 30 minutes with 40 mIU/ml oxytocin, washed, and then incubated for 60 minutes with calcium ionophore A23187, 10 μmol/L (▩, $n = 7$). Values are presented as means ± SD. Analysis of variance: $F = 19.19$, $p < 0.01$ for LTB_4; $F = 7.79$, $p < 0.01$ for LTD_4; $F = 23.47$, $p < 0.01$ for Δ-6-trans-LTB_4. *Asterisk,* Significantly lower ($p < 0.05$) than other means for each eicosanoid (Student–Newman-Keuls test).

cessing these samples as previously reported. Final recovery averaged 60% ± 5% for LTD_4 and 75% ± 6% for LTB_4 and Δ-6-trans-LTB_4.

The sensitivity of the assay for LTB_4 at 270 nm ($\epsilon = 50.000$) is 100 pg, for Δ-6-trans-LTB_4 at 270 nm ($\epsilon = 56.000$), 80 pg, and for LTD_4 at 280 nm ($\epsilon = 40.000$), 1 ng when the detector is set at 0 001 AUFS (absorbance units full scale). For LTB_4 the intraassay coefficient of variation is 4% and the interassay coefficient of variation is 3%. For LTD_4 the intraassay coefficient of variation is 5% and the interassay coefficient of variation is 8%. For Δ-6-trans-LTB_4 the intraassay coefficient of variation is 6% and the interassay coefficient of variation is 7%.

Statistical analysis. Data are presented as means ± SD. Statistical analysis was performed with a one-way analysis of variance. The Student–Newman-Keuls test was used for post hoc multiple comparisons among groups.

Results

After incubation with calcium ionophore A23187 10 μmol/L, tissues obtained after spontaneous labor released LTB_4 (2.53 ± 0.67 ng/gm wet weight per hour), LTD_4 (1.16 ± 0.58 ng/gm wet weight per hour), and Δ-6-trans-LTB_4 (1.92 ± 0.88 ng/gm wet weight per hour) into the culture medium in significantly higher amounts than did tissues obtained after elective cesarean section before labor (group 2: LTB_4, 0.40 ± 0.27 ng/gm wet weight per hour; LTD_4, 0.038 ± 0.016 ng/gm wet weight per hour; Δ-6-trans-LTB_4, nondetectable amount) (Figs. 1 and 2).

Tissues incubated in the absence of calcium ionophore (controls) released into the culture medium only minimal amounts of the previously mentioned leukotrienes, regardless of the presence or absence of labor (LTB_4, 0.11 ± 0.06 ng/gm wet weight per hour; LTD_4 and Δ-6-trans-LTB_4, nondetectable amounts).

Preincubation with oxytocin greatly increased the release into the culture medium of leukotrienes by tissues obtained in the absence of labor (LTB_4, 2.51 ± 0.47 ng/gm wet weight per hour; LTD_4, 0.73 ± 0.35 ng/gm wet weight per hour; Δ-6-trans-LTB_4, 2.37 ± 0.58 ng/gm wet weight per hour) (Figs. 1 and 2). This increase of the synthesis and release of the previously mentioned leukotrienes was statistically significant

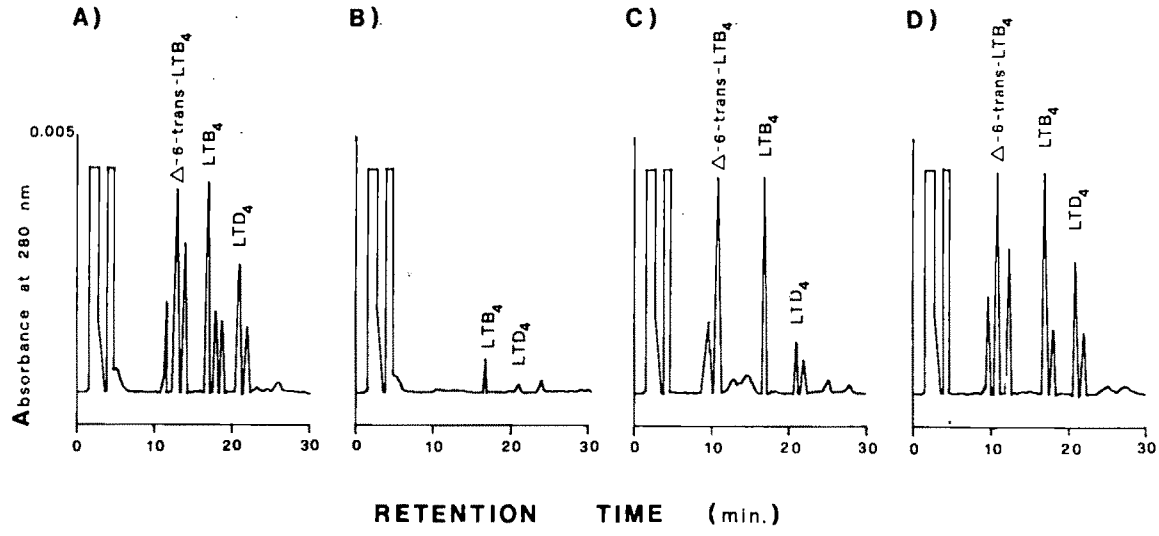


Fig. 2. Chromatographic patterns representative of culture media of human fetal membranes and uterine decidua. *A*, Vaginal delivery plus 10 μmol/L calcium ionophore A23187; *B*, cesarean section plus 10 μmol/L calcium ionophore A23187; *C*, cesarean section plus 40 mIU/ml of oxytocin plus 10 μmol/L calcium ionophore A23187; *D*, vaginal delivery plus 40 mIU/ml oxytocin plus 10 μmol/L calcium ionophore A23187. Retention times were 13, 17, and 21 minutes for Δ-6-trans-LTB_4, LTB_4, and LTD_4, respectively. Absorbance was determined at 280 nm. Attenuation setting of ultraviolet spectrophotometric detector was 0.005 AUFS.

when compared with the release by tissues obtained in absence of labor and treated only with calcium ionophore. However, when oxytocin was added to the incubation medium of tissues obtained after spontaneous labor, no additional increase of the leukotriene release was detected (LTB_4, 2.62 ± 0.98 ng/gm wet weight per hour; LTD_4, 1.18 ± 0.77 ng/gm wet weight per hour; Δ-6-trans-LTB_4, 1.97 ± 0.47 ng/gm wet weight per hour). Oxytocin stimulated leukotriene release by tissues obtained in the absence of labor in a dose-dependent fashion, the maximum effect occurred at a concentration of 40 mIU/ml (Fig. 3). Thereafter, leukotriene release by tissues markedly decreased.

Addition to culture medium of 0.5 μmol/ml of cycloheximide resulted in a marked decrease of the synthesis and release of leukotrienes by tissues obtained from subjects of group 1. This indicated that we had measured the de novo synthesis rather than a passive release of leukotrienes. The release of LTB_4 (0.85 ± 0.15 ng/gm wet weight per hour), LTD_4 (0.12 ± 0.05 ng/gm wet weight per hour), and Δ-6-trans-LTB_4 (0.015 ± 0.011 ng/gm wet weight per hour) was equivalent to mean percentage decrements of 66.40%, 89.65%, and 99.21%, respectively.

Similarly, the compound BW755c, which is a known inhibitor of both cyclooxygenase and the lipoxygenase enzymes, caused a significant reduction of the release of these compounds (LTB_4, 0.40 ± 0.08 ng/gm wet weight per hour, percentage decrement 84.20%; LTD_4, 0.040 ± 0.012 ng/gm wet weight per hour, percentage decrement 96.56%; Δ-6-trans-LTB_4, 0.520 ± 0.04 ng/gm wet weight per hour, percentage decrement 72.91%) (Fig. 4).

Comment

Our data confirm the ability of human fetal membranes and uterine decidua at term gestation to release leukotrienes in vitro, as was previously described by Rees et al.[11] Those investigators did not find a significant difference in the secretion pattern between unstimulated tissues obtained before and after labor. Results from their study are in accordance with our findings for the secretion patterns of unstimulated tissues. Our control experiments, conducted without calcium ionophore, also indicated that the basal output of LTB_4, LTD_4, and Δ-6-trans-LTB_4 was low and did not significantly change in relation to the presence of labor. These findings would argue against leukotriene production during labor.

However, when calcium ionophore A23187, which stimulates both cyclooxygenase and lipoxygenase enzymes, was added to the culture medium, leukotriene release by tissues obtained after vaginal delivery markedly increased. Confirmation that this increase resulted from an active synthesis was based on the results of

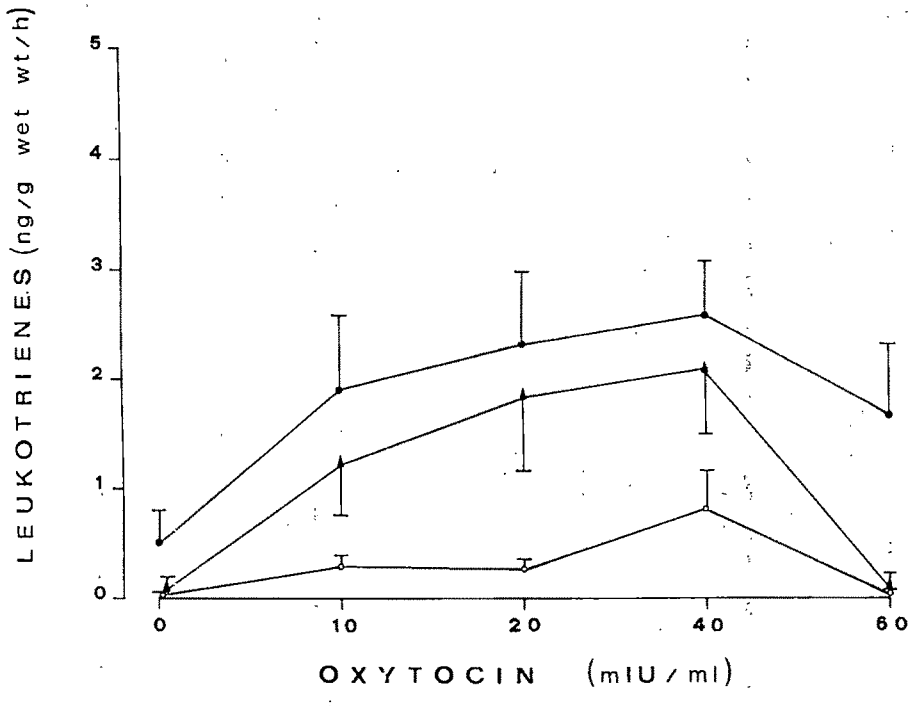


Fig. 3. Concentration-related stimulation of human fetal membranes and uterine decidua leukotriene release by oxytocin. Tissues (n = 7) were obtained during elective cesarean section before labor and were preincubated for 30 minutes with various concentrations of oxytocin, washed and then stimulated for an additional 60-minute period with 10 μmol/L calcium ionophore A23187. ●, LTB_4; ▲, Δ-6-trans-LTB_4; ○, LTD_4.

control experiments performed in the presence of cycloheximide and BW755c, which inhibit protein synthesis and arachidonic acid metabolism enzymes, respectively. The different results obtained between tissues cultured in the absence and in the presence of the calcium ionophore A23187 could be explained by suggesting that in these tissues, 5-lipoxygenase probably shows a low basal activity, and unlike other dioxygenases must be activated to transform arachidonic acid. This behavior of the 5-lipoxygenase is frequently observed in several cells and/or tissues.[12] The finding of an increased leukotriene production by samples obtained after labor and challenged with the calcium ionophore A23187 suggests that in these tissues, a 5-lipoxygenase activity is present and can be activated more easily than in tissues obtained before labor.

Thus it is possible that in human fetal membranes and uterine decidua, both the cyclooxygenase enzyme and the lipoxygenase enzymes are activated during labor. This hypothesis requires further investigation, because it is in some conflict with the initial observations that suggest an inhibition of the arachidonate lipoxygenase pathway[13] or a shift toward cyclooxygenase products during labor.[14] However, it is difficult to compare correctly the results obtained in vitro with different tissue preparations and under different experimental conditions. On the other hand, an activation of arachidonate lipoxygenase in intrauterine tissues during labor has been suggested by the increased amniotic fluid concentrations of LTB_4, the hydroxyeicosatetraenoic acids 12-HETE and 15-HETE,[5] and LTC_4[6] during human labor and of 5-HETE and LTC_4 during rhesus monkey labor.[8]

The results of this study provide evidence that oxytocin significantly stimulates the in vitro release of LTB_4, LTD_4, and Δ-6-trans-LTB_4 by human fetal membranes and uterine decidua at term gestation. This stimulatory effect was exerted at least in part in a dose-dependent fashion and was exerted only on tissues obtained after cesarean section in the absence of labor. In fact, preincubation with oxytocin did not affect the leukotrienes released by tissues obtained after spontaneous labor, probably because the receptors were already saturated by endogenous oxytocin. This assumption is further strengthened by the results of the dose-response oxytocin stimulation curve, in which leukotriene release by tissues markedly decreased at the highest concentration of oxytocin that we used.

By adding oxytocin to the culture systems of tissues taken before labor, we obtained a pattern of in vitro leukotriene release that resembled, quantitatively and qualitatively, that of tissues obtained after spontaneous

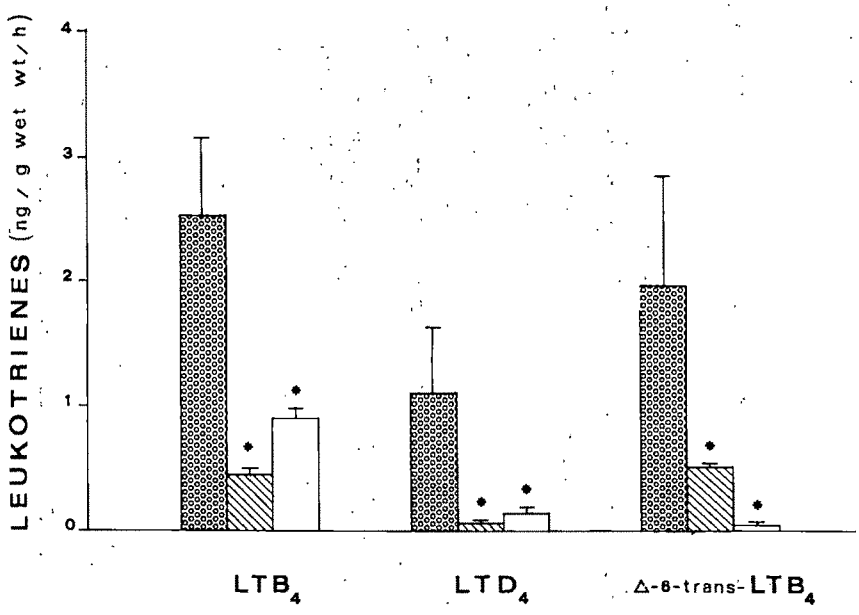


Fig. 4. Effects of BW755c and cycloheximide on leukotriene release by human fetal membranes and uterine decidua obtained after spontaneous labor at term. Tissues were divided into three specimens and treated as follows: They were stimulated for 60 minutes with 10 μmol/L calcium ionophore A23187 (▩); they were preincubated for 20 minutes with 100 μmol/L BW755c, washed, and stimulated for the subsequent 60 minutes with 10 μmol/L calcium ionophore A23187 (▨); they were coincubated with 0.5 μg/ml cycloheximide and 10 μmol/L calcium ionophore A23187 for 60 minutes (□). Data are expressed as nanograms per gram wet weight per hour. Values are the mean of seven different experiments. Analysis of variance: $F = 54.18$, $p < 0.01$ for LTB_4; $F = 25.96$, $p < 0.01$ for LTD_4; $F = 26.03$, $p < 0.01$ for Δ-6-trans-LTB_4. *Asterisk*, Significantly lower ($p < 0.05$) than control for each eicosanoid (Student–Newman-Keuls test).

labor and cultured in the absence of exogenously added oxytocin. What is the significance of this finding? Oxytocin may play a dual role in the mechanism of parturition: It is known to stimulate myometrial contractions by activating myometrial receptors and it may stimulate arachidonic acid metabolism in decidual tissues that possess specific oxytocin-binding sites,[9] which results in the formation of arachidonate lipoxygenase products.

In summary, the results of this study suggest the following conclusions: (1) The fetal membranes and uterine decidua contain an arachidonate lipoxygenase that is presumably present at a low basal activity level; nevertheless, these tissues and the placenta[15] could be an important source of amniotic and fetal circulating leukotrienes during labor; (2) the lipoxygenase activity in the tissues described is more easily inducible during labor and is pharmacologically inducible; (3) labor and oxytocin significantly affect arachidonic acid oxidative metabolism by the lipoxygenase pathway and by the cyclooxygenase pathway in the fetal membranes and uterine decidua.

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Comparison of adenocarcinoma and squamous cell carcinoma of the uterine cervix: A population-based epidemiologic study

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OBJECTIVE: Our objective was to compare epidemiologic and clinical characteristics of adenocarcinoma with those of squamous cell carcinoma of the cervix, with respect to risk by ethnic group, age at diagnosis, stage of disease at diagnosis, and survival.

STUDY DESIGN: All data were obtained from the Cancer Surveillance Program of Orange County, California, from 1984 through 1989. A total of 152 cases of adenocarcinoma and 457 of squamous cell carcinoma of the uterine cervix were included.

RESULTS: Adenocarcinoma of the cervix was diagnosed at a younger age and an earlier stage than squamous cell carcinoma. Hispanics have the highest risk for squamous cell carcinoma, whereas Asians have the highest risk for adenocarcinoma compared with whites. No differences were observed between the two histologic types in prognosis and survival.

CONCLUSION: Differences between the two histologic types of cervix cancer were found in the age at diagnosis, the extent of disease, and the ethnic distribution. In spite of these differences, prognosis and survival were not affected by histologic type. (Am J Obstet Gynecol 1992;166:1507-14.)

Key words: Adenocarcinoma of the cervix, squamous cell carcinoma of the cervix, ethnic differences, stage at diagnosis, incidence rates

The epidemiologic characteristics and clinical significance of an adenomatous histologic type in carcinoma of the uterine cervix has been the subject of considerable controversy over the past decade. During this time period changes in the incidence of adenocarcinoma of the cervix and in the proportion of cervical cancer patients with an adenomatous histologic type have been reported.[1-9] Several differences in the epidemiologic characteristics between squamous cell carcinoma and adenocarcinoma of the cervix have been shown: (1) age at diagnosis, (2) parity, (3) race, and (4) extent of disease at diagnosis.[1, 3-5, 7] Additionally, the impact of an adenomatous histologic type on the prognosis and survival of women with carcinoma of the uterine cervix has been questioned.[2-4, 6, 7]

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In spite of considerable attention to the clinical characteristics of the two histologic types of cervical cancer in the literature, no consistency in conclusions exists. One major reason for the lack of agreement is the difference in methods used in patient selection in these studies. The majority of these reports are based on studies of patients from single institutions, with the use of retrospective data. As a result, they may suffer from selection bias.

The purpose of this cohort study is to determine the epidemiologic and clinical characteristics of adenocarcinoma and to compare them with characteristics of squamous cell carcinoma of the cervix, particularly with respect to population risk by ethnic group, age at diagnosis, stage of disease at diagnosis, and survival.

Material and methods

Study subjects. The current study is based on 678 patients with histologically confirmed invasive cervical cancer diagnosed between 1984 and 1989 and included as a part of a population-based cancer registry, the Cancer Surveillance Program of Orange County. This program is one of 10 regional registries constituting the California cancer reporting system, meeting all reporting requirements of the Surveillance, Epidemiology, and End Results program of the National Cancer Institute. Description of the Cancer Surveillance Program of Orange County and details of data collection methods have been reported previously.[10] Standard data items for each case were abstracted from medical

Table I. Number of cases of invasive cervical cancer in Orange County residents diagnosed 1984 through 1989 by histology grouping (ICD-O morphology code)

		No.	*%*
Squamous			
8070	Squamous cell carcinoma	279	
8071	Squamous cell, keratinizing	59	
8072	Squamous cell, nonkeratinizing, large cell	70	
8076	Squamous cell carcinoma, microinvasive	49	
		457	67.4
Adenocarcinoma			
8140	Adenocarcinoma	81	
8260	Papillary adenocarcinoma	11	
8430	Mucoepidermoid carcinoma	1	
8441	Serous adenocarcinoma	1	
8480	Mucinous adenocarcinoma	1	
8481	Mucin-producing adenocarcinoma	4	
8560	Adenosquamous carcinoma	53	
		152	22.4
Other histologic types		69	10.2
TOTAL		678	100

records and pathology reports. Data quality for the registry is maintained through periodic training programs for hospital registrars and field abstractors, extensive visual and computer editing, and reabstraction of a 10% sample of cases from each facility reporting to the registry.

As a population-based cancer registry the Cancer Surveillance Program of Orange County maintains a cancer information reporting system that includes case reports for all cancer patients seen in every hospital in Orange County and case reports for Orange County resident patients diagnosed or treated for active cancer at hospitals outside the county. Also included are case reports from physicians' offices and other facilities in Orange County, such as clinics where cancer may be diagnosed and treated without hospitalization of the patient. To ensure that all cases of cancer in Orange County residents are included in the Cancer Surveillance Program of Orange County information system, death certificates of county residents with cancer reported as cause of death are matched to case reports on file. Where no case report exists, the source certifying the death is contacted for more information, and a case report is generated for the patient. Non–Orange County residents with diagnosis and treatment in Orange County referral facilities are not included in the patient group under study.

Data taken from Cancer Surveillance Program of Orange County include demographic information (birth date, age, sex, ethnicity, etc.), laboratory data, pathology studies, and stage of disease at time of diagnosis. These data were abstracted from medical and pathology records by trained tumor registrars according to standard procedures.[11] Tumor site and histologic classification were coded according to the International Classification of Diseases for Oncology (ICD-O), published by the World Health Organization.[12]

The 678 individuals studied here with primary invasive cervical cancers include all of the Orange County residents with this malignancy reported to Cancer Surveillance Program of Orange County by April 1991 for the years 1984 through 1989. All cases included in this study were histologically confirmed primary invasive cervical cancers (ICD-O codes 180.0 through 180.9). Specific histology codes were grouped into cell types with World Health Organization specifications for typing of cervical cancer: ICD-O codes 8070 through 8072 and 8076 were grouped as squamous cell carcinoma (457 cases); codes 8140, 8260, 8430, 8441, 8480, 8481, and 8560 were adenocarcinoma (152 cases); other histologic types totaled 69. Among the cases having cancers of other histologic types, there were 42 with either carcinoma, not otherwise specified (ICD-O code 8010) or malignant neoplasm (ICD-O code 8000), and the remaining 27 had cervical cancer of other histologic types (each of those histologic types contained a maximum of four cases). The frequency distribution of cases by histologic type is presented in Table I. Because the current study focuses on the comparison between adenocarcinoma and squamous cell carcinoma, the results presented here include only cases with one of these two histologic types (a total of 609 cases). Patients were also classified as to stage of disease at diagnosis according to the National Cancer Institute and the American College of Surgeons staging system into localized (equivalent to International Federation of Gynecology and Obstetrics stages IA and IB combined), regional (equivalent to International Federation of Gynecology and Obstetrics stages IIA, IIB, IIIA, and IIIB combined), and distant (equivalent to International Federation of Gynecology and Obstetrics stages IVA and IVB combined).

The ethnic distribution for squamous cell carcinoma of the cervix was 302 non-Hispanic whites (66.1%), 100 Hispanics (21.9%), 37 Asians and Pacific Islanders (8.1%), seven blacks (1.5%), and 11 other and unknown (2.4%). For adenocarcinoma the ethnic distribution was 103 non-Hispanic whites (67.8%), 28 Hispanics (18.4%), 17 Asians and Pacific Islanders (11.2%), four other and unknown (2.6%), and no blacks. Because of the small number of cases of black or other and unknown ethnicity, the data by ethnicity will be presented only for non-Hispanic whites, Hispanics, and Asians and Pacific Islanders.

The 1980 population of Orange County reported by the United States Census included 954,000 male residents with a median age of 28.5 years and 979,000

female residents with a median age of 30.5 years. Postcensus figures for Orange County computed by the Forecasting and Analysis Center of the Orange County Administrative Office (Anton-Culver H, et al. University of California, Irvine, unpublished observations) estimated the 1984 population at 2.1 million, which included 73% whites, 17% Hispanics, 7% Asians and Pacific Islanders, and 1% non-Hispanic blacks. The total county population reported by the 1990 census was 2.4 million, 64.5% of whom were whites, 23.4% Hispanics, 10.0% Asians and Pacific Islanders, and 1.6% non-Hispanic blacks.[13]

Analysis. Age-specific incidence rates for cancer of the cervix were calculated with population estimates determined by the Orange County Administrative Offices. Age-adjusted incidence rates were calculated by the direct adjustment method, with the 1970 United States population used as a standard. Statistical analyses were carried out with BMDP[14] and SAS[15] statistical software packages.

Survival of patients with each of two histologic types of invasive cervical cancer, adenocarcinoma and squamous cell, was compared by means of the log rank test[16] as computed with the SAS procedure LIFETEST.[15]

Results

Age-adjusted incidence rates and ethnic groups. The 6-year average age-adjusted incidence rates of squamous cell carcinoma and adenocarcinoma of the cervix by ethnic group are given in Table II. It is clear that for squamous cell carcinoma the risk is higher in both Hispanics and Asians than in non-Hispanic whites (11.2, 9.5, and 5.0 per 100,000 population, respectively). The incidence of adenocarcinoma is highest in Asians and Pacific Islanders, intermediate for Hispanics, and lowest in non-Hispanic whites (4.0, 2.7, and 1.7, respectively).

For all ethnic groups combined the standardized risk ratio comparing age-adjusted incidence rates for squamous cell carcinoma with rates for adenocarcinoma is 3.07. Among both Asians and non-Hispanic whites the standardized risk ratio comparing the age-adjusted incidence rate for squamous cell carcinoma with that of adenocarcinoma is similar (2.4 to 2.9, respectively). The standardized risk ratio comparing squamous cell carcinoma with adenocarcinoma of the cervix among Hispanics (4.2) is numerically greater than in the other two ethnic groups.

Age distribution and age-specific rates by ethnicity. The age-specific rates for squamous cell carcinoma range from 1.66 per 100,000 for the age group 20 to 24 to a maximum of 16.01 per 100,000 at age group 65 to 69. For adenocarcinoma the age-specific incidence rates range from 0.18 per 100,000 for age group 20 to 24 to a high of 5.5 per 100,000 for age group 40 to 44.

Table II. Invasive cancer of the cervix in Orange County residents diagnosed between 1984 and 1989: Mean age, median age, age-adjusted incidence rates,* and frequency by histologic type and ethnicity for cervical cancer patients

Ethnicity	*Histologic type*	
	Squamous cell carcinoma	*Adenocarcinoma*
White		
Mean age (yr)	52.3	47.84
Median age (yr)	50	44
Age-adjusted incidence rate	5.00	1.72
No.	302 (66.1%)	103 (67.8%)
Hispanic		
Mean age (yr)	47.3	44.46
Median age (yr)	46	41
Age-adjusted incidence rate	11.20	2.68
No.	100 (21.9%)	28 (18.4%)
Asian and Pacific Islander		
Mean (age (yr)	52.62	49.06
Median age (yr)	52	47
Age-adjuster incidence rate	9.54	4.01
No.	37 (8.1%)	17 (11.2%)
All patients		
Mean age (yr)	50.85	47.31
Median age (yr)	48	44
Age-adjuster incidence rate	5.91	1.92
No.	457 (100%)	152 (100%)

*Six-year average age-adjusted incidence rates with 1987 female population used as denominator.

In the various age groups the age-specific incidence rates for squamous cell carcinoma of the cervix generally are of the order of 2 to 6.5 times the incidence rates for adenocarcinoma.

The cumulative annual age-specific incidence rates of adenocarcinoma and squamous cell carcinoma of the cervix are given in Fig. 1, *A*. The cumulative rates increase with age for both histologic types. However, the observed ratio of the cumulative rates for squamous cell carcinoma to the cumulative rates for adenocarcinoma increases with age. For adenocarcinoma the cumulative age-specific incidence rates among both Hispanics and Asians and Pacific Islanders are higher in all age groups above 45 (Fig. 1, *B*). It also appears that Asians and Pacific Islanders have the highest cumulative incidence rates for adenocarcinoma at all age groups above 45. For squamous cell carcinoma, above the age of 45 both Hispanics and Asians and Pacific Islanders have cumulative age-specific rates that are consistently higher than the corresponding rates for non-Hispanic whites (Fig. 1, *C*). However, Hispanics appear to have the highest cumulative incidence for

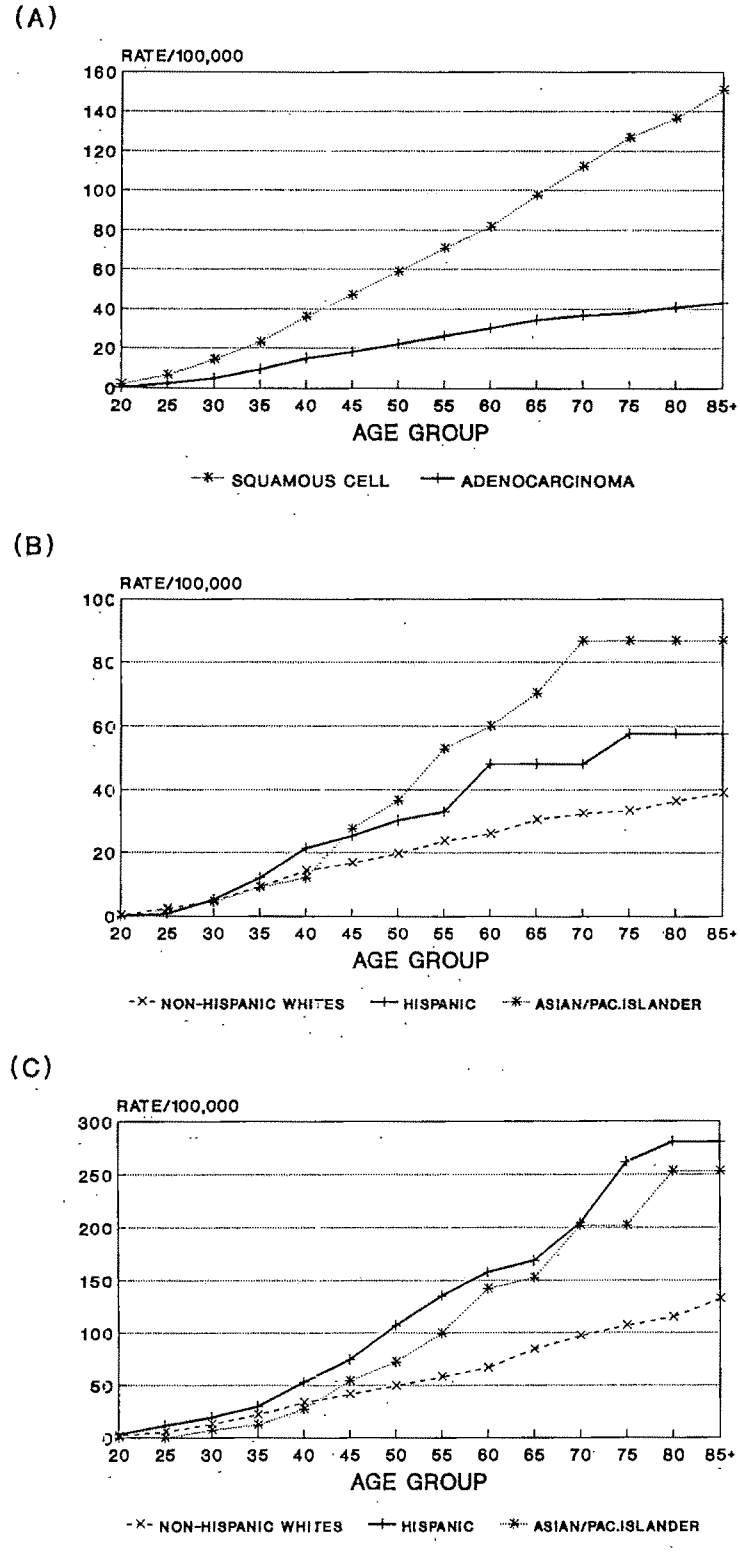


Fig. 1. Cumulative annual age-specific incidence rates per 100,000 of cervical cancer by histologic type **(A)**, by ethnic group for adenocarcinoma **(B)**, and by ethnic group for squamous cell carcinoma **(C)**.

squamous cell carcinoma, in contrast to adenocarcinoma, at all ages.

Stage at diagnosis and histologic type. The data on the distribution of cases by summary stage at diagnosis (localized, regional, and distant disease involvement) and histologic type are presented in Table III. For patients with squamous cell carcinoma, 45.7% had localized disease at diagnosis compared with 58.6% for patients with adenocarcinoma (Table III). At diagnosis 4.4% of patients with squamous cell carcinoma and

Table III. Distribution of summary stage in cervical cancer patients by histologic type

	Histologic type					
	Squamous cell carcinoma		*Adenocarcinoma*		*Total*	
Stage	*No.*	*%*	*No.*	*%*	*No.*	*%*
Local	209	45.7	89	58.6	298	48.9
Regional	174	38.1	41	27.0	215	35.3
Distant	54	11.8	18	11.8	72	11.8
Unknown	20	4.4	4	2.6	24	3.9
TOTAL	457	100	152	100	609	100

Table IV. Distribution of summary stage in cervical cancer patients by age group and histologic type

		Histologic type					
		Squamous cell carcinoma		*Adenocarcinoma*		*Total*	
Age (yr)	*Stage*	*No.*	*%*	*No.*	*%*	*No.*	*%*
<40	Local	89	67.9	36	66.7	125	67.6
	Regional	29	22.1	14	25.9	43	23.2
	Distant	13	9.9	4	7.4	17	9.2
	Total	131	100	54	100	185	100
40-54	Local	62	45.6	35	70.0	97	52.2
	Regional	63	46.3	12	24.0	75	40.3
	Distant	11	8.1	3	6.0	14	7.5
	Total	136	100	50	100	186	100
≥55	Local	58	34.1	18	40.9	76	35.5
	Regional	82	48.2	15	34.1	97	45.3
	Distant	30	17.6	11	25.0	14	19.2
	Total	170	100	44	100	214	100

2.6% of patients with adenocarcinoma had unknown stage. The distribution of squamous cell carcinoma and adenocarcinoma by stage at diagnosis is significantly different ($p < 0.05$). When cases were classified further by age groups (Table IV), the distribution by stage at diagnosis differed significantly between squamous cell carcinoma and adenocarcinoma only for the age group 40 to 54 years; 70.0% of cases of adenocarcinoma were diagnosed at the localized stage, compared with 45.6% of cases of squamous cell carcinoma ($p = 0.05$) (Table IV). There were no significant differences in stage at diagnosis for the two cell types in the remaining age groups, <40 and ≥55. The distribution of cases by stage at diagnosis and histologic type is examined further by ethnicity in Table V. In each ethnic group the trend is toward less aggressive disease in adenocarcinoma than in squamous cell carcinoma, although the differences are not statistically significant.

Survival by histologic type. The survival in cases of adenocarcinoma and squamous cell carcinoma of the cervix is presented in Fig. 2, *A*. After adjustment for differences in age at diagnosis, the survival of the two groups defined by histologic type was not significantly different ($p = 0.76$). Fig. 2, *B* and *C*, presents the survival by stage at diagnosis for adenocarcinoma and squamous cell carcinoma of the cervix, respectively. For both cell types the survival differed significantly by stage at diagnosis ($p < 0.001$). After adjustment for both age and stage at diagnosis the survival of the two histologic groups was not significantly different ($p = 0.87$).

Comment

The population sample studied was 678 patients with invasive histologically confirmed cervical cancer, diagnosed during the 6-year period 1984 through 1989 and identified as a part of the population-based cancer registry, the Cancer Surveillance Program of Orange County. Of all cases studied, 457 (67.4%) were squamous cell carcinoma and 152 (22.4%) were adenocarcinoma. In this study differences between the two histologic types are shown with respect to age-adjusted incidence rates, age-specific rates, age-adjusted and age-specific rates classified by ethnic group, distribution by stage at diagnosis, and survival with adjustment for stage at diagnosis and age.

The proportion of adenocarcinoma found in this study (22.4%) is higher than that reported by previous investigators.[2-4, 6, 7, 9] Horowitz et al.[1] reported a study of 333 patients with cervical cancer diagnosed at Johns Hopkins, 16% of which cases were adenocarcinoma. These authors studied differences in demographic and

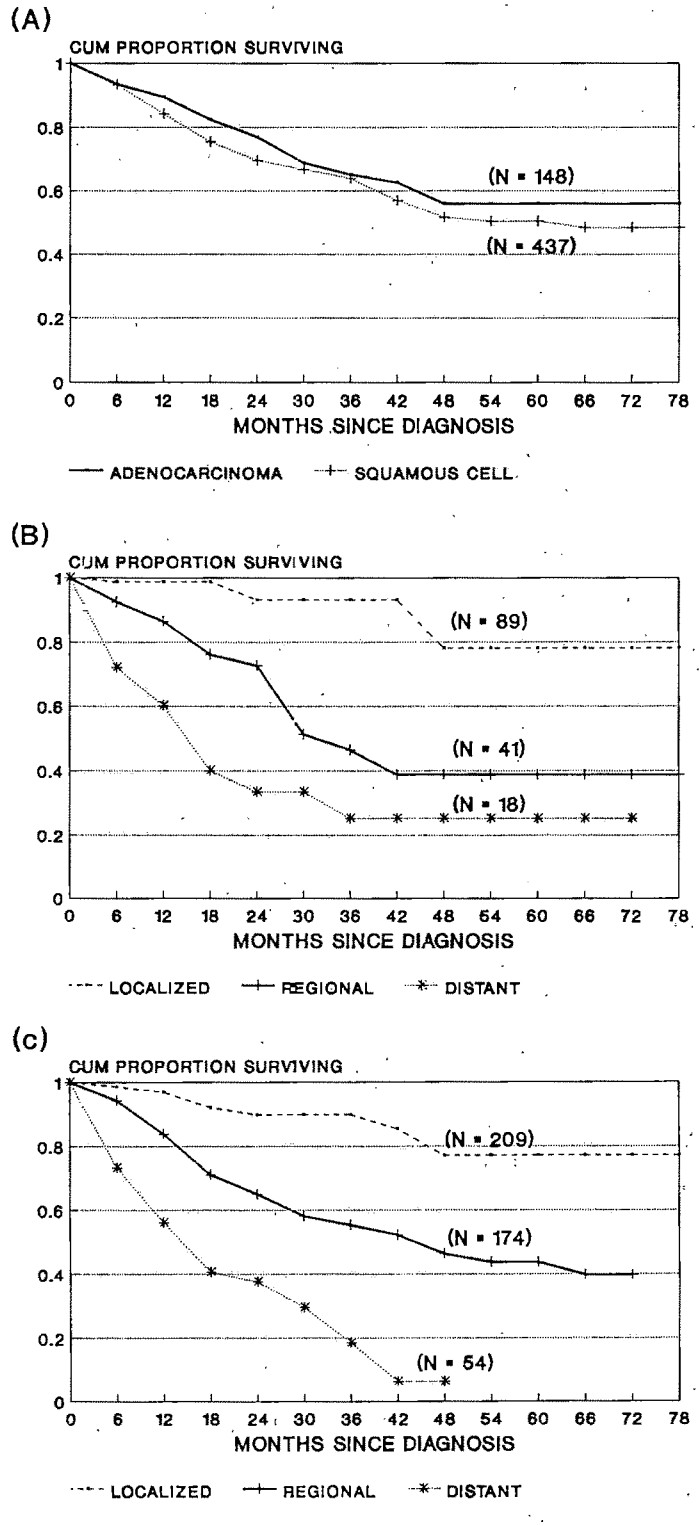


Fig. 2. Cumulative survival of patients with cervical cancer diagnosed in 1984 through 1989 by histologic type **(A)**, by stage at diagnosis for adenocarcinoma **(B)**, and by stage at diagnosis for squamous cell carcinoma **(C)**.

socioeconomic factors between the two histologic groups, concluding that their "data suggest that epidermoid and adenocarcinoma of the cervix may represent diseases with distinct populations at risk."[1] Silcocks et al.[3] report a study of squamous cell and adenocarcinoma of the cervix with data from the South Thames cancer registry, showing only 13.2% of all invasive carcinoma of the cervix to be of adenomatous histologic type. Several other reports suggest that the proportion of adenocarcinoma of the cervix is increas-

Table V. Distribution of summary stage in cervical cancer patients by histologic type and ethnicity

Ethnicity	Stage	Histologic type				Total	
		Squamous cell carcinoma		Adenocarcinoma			
		No.	%	No.	%	No.	%
White	Local	146	48.3	60	58.3	206	50.9
	Regional	107	35.4	28	27.2	135	33.3
	Distant	36	11.9	13	12.6	49	12.1
	Unknown	13	4.3	2	1.9	15	3.7
	Total	302	100	103	100	405	100
Hispanic	Local	41	41.0	18	64.3	59	46.1
	Regional	40	40.0	6	21.4	46	35.9
	Distant	13	13.0	4	14.3	17	13.3
	Unknown	6	6.0	0	0.0	6	4.7
	Total	100	100	28	100	128	100
Asian and Pacific Islander	Local	13	35.1	8	47.1	21	38.9
	Regional	22	59.5	7	41.2	29	53.7
	Distant	2	5.4	1	5.9	3	5.6
	Unknown	0	0.0	1	5.9	1	1.9
	Total	37	100	17	100	54	100

ing over time.[1, 2, 8, 9] The proportions reported in these studies range from 7% to 20%.

The difference in the proportion of adenocarcinoma reported in this study may well be a result of the variability in the ethnic distribution of the population at risk. None of the previously reported studies present data on incidence rate or distribution of histologic types by ethnic group; the data on the ethnic distribution of the population at risk in these studies were not reported. The data from the present study indicate differences among ethnic groups with regard to the risk for adenocarcinoma and squamous cell carcinoma and to differences in the stage at diagnosis.

Hispanics appear to be at highest risk for squamous cell carcinoma at all ages, followed by Asians and Pacific Islanders and then non-Hispanic whites. For adenocarcinoma Asians and Pacific Islanders have the highest age-adjusted incidence rates, followed by Hispanics and again non-Hispanic whites with the lowest rates (Table II). For the three ethnic groups combined, the risk for squamous cell carcinoma is highest (16.1/100,000) at age 65 to 69, whereas in adenocarcinoma the incidence rate is highest (5.5/100,000) for age group 40 to 44. Furthermore, 45.7% of squamous cell carcinomas are diagnosed at a localized stage, compared with 58.6% of adenocarcinomas. The distribution by stage of diagnosis of the two histologic types is significantly different ($p < 0.05$) (Tables III and IV).

The data from the current study, with respect to incidence rate, age at diagnosis, and stage of diagnosis for each of the three ethnic groups, indicate that adenocarcinoma is less aggressive than squamous cell carcinoma (Table V). In general, adenocarcinomas were diagnosed at an earlier stage than squamous cell carcinomas regardless of age and ethnicity. These differences are most pronounced in women aged 40 to 54 and in Hispanic women. This finding is directly opposite to the popular belief that adenocarcinomas are usually more advanced than squamous cell carcinomas at the time of diagnosis. A generally accepted rationale for this belief is that adenocarcinoma occurs high in the endocervical canal and is therefore hidden from early diagnosis by inspection or Papanicolaou smear.

Within each of the two groups defined by histologic type, survival is significantly different by stage at diagnosis. As expected, survival is inversely related to the advancement of the disease at diagnosis, which is in general agreement with earlier reports of survival.[2, 4, 6, 7, 17, 18] In this study survival did not differ by histologic type when adjusted for stage at diagnosis and age. This finding differs from conclusions of other investigators who have suggested that adenocarcinoma carries a worse prognosis.

In conclusion, we have compared the epidemiologic and clinical behavior of adenocarcinoma with that of squamous cell carcinoma of the uterine cervix with a population-based cohort. Major differences between the two histologic types were found in the age at diagnosis (adenocarcinoma earlier than squamous cell carcinoma), the extent of disease at diagnosis (adenocarcinoma less advanced than squamous cell carcinoma), and differences in the age-adjusted incidence rate with regard to ethnic distribution. In spite of these epidemiologic differences, prognosis and survival were not affected by histologic type.

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A review of premature birth and subclinical infection

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Premature birth causes high rates of neonatal morbidity and mortality. There are multiple causes of preterm birth. This article reviews the evidence linking subclinical infection and premature birth. Although maternal genital tract colonization with specific organisms has been inconsistently associated with preterm birth and/or premature rupture of membranes, some infections have been consistently associated with preterm delivery. The association of histologic chorioamnionitis with prematurity is a consistent finding, but the mechanisms require further study. The relationship between histologic chorioamnionitis infection and the chorioamnionitis of prematurity requires additional research. A varying number of patients in "idiopathic" preterm labor have positive amniotic fluid cultures (0% to 30%), but it is not clear whether infection preceded labor or occurred as a result of labor. Evidence of subclinical infection as a cause of preterm labor is raised by finding elevated maternal serum C-reactive protein and abnormal amniotic fluid organic acid levels in some patients in preterm labor. Biochemical mechanisms for preterm labor in the setting of infection are suggested by both in vitro and in vivo studies of prostaglandins and their metabolites, endotoxin and cytokines. Some, but by no means all, antibiotic trials conducted to date have reported decreases in prematurity. These results support the hypothesis that premature birth results in part from infection caused by genital tract bacteria. In the next few years, research efforts must be prioritized to determine the role of infection and the appropriate prevention of this cause of prematurity. (AM J OBSTET GYNECOL 1992;166:1515-28.)

Key words: Premature birth, subclinical infection, biochemical mechanisms, chorioamnionitis, antibiotics

Preterm birth is the leading perinatal problem in the United States. The underlying cause of preterm birth is not evident in most cases, and currently, physicians are left with marginally effective attempts to delay delivery by arresting contractions. Breakthroughs in the prevention of preterm birth will require an improved understanding of its causes followed by appropriate strategies to prevent these causes. Evidence from several sources suggests a link between subclinical infection and prematurity. In August 1990 a symposium, sponsored by the Infectious Disease Society of Obstetrics and Gynecology, was held to discuss this evidence. This article summarizes the presentations and includes additional data presented since the symposium.

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Prematurity in perspective

The problems of being born premature or with low birth weight (LBW) are well documented. LBW leads to excessive neonatal mortality and morbidity. More than 60% of the mortality that occurs among infants without anatomic or chromosomal congenital defects is attributable to LBW.[1] A similar excess of morbidity also exists in the LBW group. The Surgeon General placed a high priority on the reduction of LBW by 1990. However, the rate of LBW has not appreciably changed in the United States over the past 40 years.[2] Approximately 6.7% of infants are born with LBW and 1% are born with very low birth weight (<1500 gm).[3] The United States ranks nineteenth in the world in infant mortality, behind virtually all European countries and in the same range as many third-world countries.

In contrast, the neonatal mortality rate has been dramatically reduced over the same 40 years in the United States,[4] caused mainly by improved care of LBW infants in neonatal intensive care units.[2] In fact, weight for

weight, an infant has the best survival rate in the United States. Thus, given a high LBW rate, infant mortality would be even higher in the United States were it not for high technology and expensive neonatal care. It is apparent that the emphasis of medical care for this problem in the United States has been placed on costly tertiary care rather than on prevention.

The main reason for a continued high LBW rate is a poor understanding of antenatal factors that contribute to an infant with LBW. One cannot expect to reduce LBW or even to appreciate the relative importance of various potential factors until the various causes are determined. Most investigators have assumed a final common pathway by which a variety of normal and pathologic processes eventually influence the human endocrinologic system that, in turn, causes uterine contractions and labor. While parts of this system have been identified in certain animals, processes important in human parturition are less clear. Without additional knowledge on the essential pathway, it has been difficult to identify the mechanisms by which various processes result in premature contractions or delivery.

To date, multiple factors have been associated with LBW. Determination of factors that are important causes of LBW awaits further study. Some factors appear highly associated with infant mortality and LBW because of consistent findings, a high relative risk ratio, or a high frequency of the factor in the population. Risk factors for both neonatal and postneonatal mortality include both low (<17 years) and high (>34 years) maternal age, black race, and low socioeconomic status.[2] These same three factors plus a history of an adverse pregnancy outcome, inadequate prenatal care, rupture of membranes, multiple pregnancies, medical conditions such as hypertension and diabetes, fetal anomalies, and drug abuse are some of the more important factors associated with LBW.[2] While some of these factors cause premature delivery, others such as socioeconomic status are only associated with preterm delivery.

Premature rupture of membranes is associated with about one third of LBW deliveries. Premature rupture of membranes has been reported to be the third leading cause of perinatal death in the collaborative perinatal project after "amniotic fluid infection" and abruptio placentae.[5] Factors associated with premature rupture of membranes include premature rupture of membranes in a prior pregnancy,[6] histologic chorioamnionitis,[7] abnormal bleeding, polyhydramnios, incompetent cervix, smoking,[8] and selected vaginal microorganisms[9] in the current pregnancy. Infection appears to be an important theme in premature rupture of membranes.

Recently, convincing evidence has associated LBW with infection. High rates of amniotic fluid infection, chorioamnion infection, and histologic chorioamnionitis have been reported in LBW pregnancies.[10] The highest proportion of these three infections occurs in the most premature pregnancies, an observation that adds to the impact of infection in LBW.

Prevention of LBW and premature rupture of membranes can be targeted (1) to prevent the initiation of labor or premature rupture of membranes or (2) to inhibit preterm labor after it is recognized. Programs for prevention of preterm labor have had only limited success. The targeted treatment of cervical or vaginal infection has led to only a modest reduction of preterm labor and premature rupture of membranes, and the overall impact on preterm labor may be limited. Treatment of premature labor with tocolysis also has had limited impact on LBW and premature rupture of membranes.

Experimental difficulties exist in establishing the role of infection in LBW and premature rupture of membranes. Many LBW deliveries occur without placental or amniotic fluid infection. Antibiotic treatment of uninfected pregnancies would have no effect and inclusion of uninfected pregnancies into such treatment studies will usually mask any positive antibiotic effect. The detection of infection requires sensitive techniques because most maternal infection is subclinical. Upper genital tract infection most directly influences preterm delivery and sampling of the upper genital tract is difficult, particularly before premature labor. Whereas amniotic fluid can be sampled before delivery, the majority of infections occur in the placenta and currently it is not possible to sample the placenta before delivery. A cause-and-effect relationship is even harder to establish after an event has transpired.

Specific microorganisms associated with preterm birth

The first evidence that suggested a connection between infection and premature birth occurred with an association of a variety of cervicovaginal microorganisms with preterm delivery, LBW, or premature rupture of membranes (Table I). The next question was whether those lower maternal genital tract isolates were related to upper genital tract infection and, further, whether the relationship between upper genital tract infection and preterm birth, LBW, and premature rupture of membranes was a cause or an effect.

Syphilis has been related to preterm birth for many years. Untreated primary or secondary syphilis resulted in a 50% rate of prematurity compared with an 8% rate among controls.[11] Early latent syphilis resulted in a 20% prematurity rate. The association of syphilis with premature delivery has been reconfirmed during the current epidemic of congenital syphilis in which mean gestational age for newborns with congenital syphilis was 32.3 weeks. Twelve (39%) of 33 infants with congenital

syphilis and none of the controls had premature rupture of membranes.[12]

Untreated *N. gonorrhoeae* has been associated with preterm delivery.[13-16, 16A] In addition, treatment of *N. gonorrhoeae* reduces rates of preterm delivery and low birth weight similar to that of control populations, suggesting a causative role of *N. gonorrhoeae* in these events.

Asymptomatic group B streptococci bacteriuria has been consistently associated with preterm delivery. Preterm delivery rates of 20% versus 8.5%[17] and 7.2% versus 4.9%[18] occurred among women with bacteriuria with and without group B streptococci, respectively. A sevenfold decrease in preterm delivery resulted when antibiotics were administered (5.4%), compared with placebo (37.5%).[19] The extraordinarily high rate of prematurity in the bacteriuric group suggests that additional risk factors for prematurity may have been present.

In comparison to the consistent association with bacteriuria, the relationship of maternal group B streptococci genital colonization and adverse pregnancy outcome has been inconsistent. Five of the six studies assessing genital tract group B streptococci colonization with preterm labor or delivery demonstrated no significant association.[20-25] However, a significant association was found in three of four studies assessing the relationship of genital group B streptococci colonization and in preterm premature rupture of membranes[21, 22, 26, 27] (Table II).

In case-control studies genital tract mycoplasmas, especially *U. urealyticum*, have been associated with LBW.[28, 29] A subgroup of pregnant women with serologic evidence of invasive *U. urealyticum* infection (more than a fourfold rise in antibody) has been associated with LBW.[30] The mean birth weight was 233 gm lower in the group with an antibody response (3006 ± 565 vs 3229 ± 474 gm, $p = 0.009$). The clinical significance of a 200 gm difference at a 3 kg birth weight is questionable. A larger proportion of newborns weighing <2500 gm were born to mothers with a fourfold rise in antibody to *U. urealyticum* (38%, 10/27) compared with infants weighing >2500 gm (15%, 33/219). However, recent studies have failed to confirm such an association. None of the 11 cohort studies performed[31-41] support an association between *U. urealyticum* and prematurity or LBW. The treatment of *U. urealyticum* with erythromycin had no impact on LBW or premature rupture of membranes.[42] *M. hominis* has been inconsistently related with LBW; the relationship has been positive in some[25, 26, 31, 38, 42A] but not other[28, 35, 38] studies. Thus the majority of investigations have failed to provide evidence supporting an association between colonization of the maternal genital tract with *U. urealyticum* and preterm delivery or LBW. Data regarding *M. hominis* are less clear in relating this organism to prematurity.

Table I. Microorganisms or infections associated with preterm labor, preterm delivery, and preterm premature rupture of the membranes

Organisms
Treponema pallidum
Neisseria gonorrhoeae
Group B streptococci
Ureaplasma urealyticum
Mycoplasma hominis
Chlamydia trachomatis
Trichomonas vaginalis
Bacteroides sp.
Infections
Bacterial vaginosis
Pyelonephritis
Bacteriuria

The presence of *C. trachomatis* in the cervix at the initial prenatal visit was associated with spontaneous abortion, stillbirth, preterm delivery, and LBW.[38] The mean gestational age was 35.9 weeks in those with and 39.2 weeks in those without *C. trachomatis* ($p < 0.01$).[38] LBW was also associated with *C. trachomatis* in this study. Subsequent studies by Thompson et al.,[39] Harrison et al.,[31] and Hardy et al.[40] failed to confirm an overall association between chlamydia infection and adverse pregnancy outcome. However, Harrison et al. reported that a subgroup of women with cervical *C. trachomatis* and elevated levels of IgM antibody against *C. trachomatis* (suggestive of recent acquistion or active infection) had a significantly increased risk of premature rupture of membranes, LBW, and preterm delivery.[31] Sweet et al.[35] confirmed these findings. After controlling for the presence of other sexually transmitted disease organisms in a multivariate analysis, the association was no longer significant.[35] In another investigation *C. trachomatis* remained associated with LBW and premature rupture of membranes in a multivariate analysis controlling for bacterial vaginosis and obstetric factors related to prematurity.[43] Several other recent investigations support an association of *C. trachomatis* with preterm delivery.[25, 26, 37, 42A] Thus further large-scale studies that also use serologic assessment will be required to resolve the controversy over the role of *C. trachomatis* in preterm delivery and premature rupture of membranes.

The association of *T. vaginalis* with preterm delivery has also been a source of controversy. *T. vaginalis* was not associated with LBW,[44] and treatment was not associated with an increase in birth weight or gestational age in a placebo-controlled trial.[45] However, among adolescents, the incidence of LBW was 18% in patients with versus 6.7% in those without *T. vaginalis*

Table II. Genital tract colonization of group B streptococci and preterm delivery

Series	*Preterm labor*		*Preterm delivery*		*Premature rupture of membranes*	
	GBS pos. (%)	*GBS neg. (%)*	*GBS pos. (%)*	*GBS neg. (%)*	*GBS pos. (%)*	*GBS neg. (%)*
Baker et al.[20]	11	6.3	–	–	–	–
Regan et al.[21]	–	–	5.4	1.26	15.3	7*
Minkoff et al.[22]	17	8.7	–	–	10	8.8*
Hastings et al.[23]	–	–	6.5	6.4	–	–
Bobitt et al.[26]	–	–	–	–	5.6	1.7*
Lamont et al.[24]	6	0	–	–	–	–
Martius et al.[25]	21	11	–	–	–	–
Alger et al.[27]	–	–	–	–	16	4*

GBS, Group B streptococci.
*Statistically significant difference.

($p = 0.06$).[40] Further, in the large Vaginal Infections and Prematurity Study, *T. vaginalis* carriage at midpregnancy was significantly associated with LBW and premature rupture of membranes after adjustment for confounding factors and other microorganisms (Cotch MF. Personal communication, ICAAC abstracts, 1990). Finally, the incidence of premature rupture of membranes at term was 27.5% in women with positive cultures compared with 12.8% in those with negative cultures for *T. vaginalis* ($p < 0.03$).[22]

Bacteroides sp. have also been implicated in preterm delivery and/or premature rupture of membranes. In a stepwise logistic regression analysis, women colonized with *Bacteroides sp.* at their initial prenatal visit had a 40% increased preterm delivery rate compared with that for noncolonized women ($p = 0.003$).[22] Nonfragilis *Bacteroides sp.* were more often recovered from the cervices of women in spontaneous preterm labor (21% of 72) compared with those women not in labor but at a similar gestational age (4% of 26, $p = 0.03$).[25] Black-pigmented *Bacteroides sp.* were more frequently recovered from women in preterm labor who were delivered at <37 weeks (20% of 61) compared with control women at term (8% of 155, $p < 0.05$).[26] More recently, vaginal *Bacteroides sp.* has been associated with a 60% increased risk of preterm delivery, and *Bacteroides bivius* $>10^4$ colony-forming units per milliliter was associated with a twofold increased risk of delivery at ≤34 weeks' gestation.[46, 47]

Bacterial vaginosis is a clinical condition in which the normal vaginal flora, characterized by high concentrations of lactobacilli, is replaced by high concentrations of anaerobic bacteria, especially *Bacteroides sp.* and *Mobiluncus sp.*, *Gardnerella vaginalis*, and *M. hominis*.[48] Several studies have suggested that bacterial vaginosis is associated with LBW and premature rupture of membranes.[10, 26, 43, 49] Premature rupture of membranes occurred significantly more commonly in the bacterial vaginosis group (46%) than in those without bacterial vaginosis (4%).[49] In a cohort of pregnant women, bacterial vaginosis in the second or third trimesters of pregnancy was associated with LBW and premature rupture of membranes by univariate analysis. Bacterial vaginosis remained significantly associated with preterm labor (odds ratio 2.0, $p < 0.05$) and preterm premature rupture of membranes (odds ratio 3.0, $p = 0.03$) in a multivariate analysis controlling for *C. trachomatis* and risk factors for premature delivery. No significant association was present between bacterial vaginosis and LBW in the multivariate analyses. In other studies, bacterial vaginosis with preterm labor[43] or preterm delivery[26] with an odds ratio of 2 to 3. In the only cohort study in which patients were enrolled early in pregnancy (first prenatal visit), bacterial vaginosis was associated with a trend for an association with preterm labor (50% vs 29%), but these results were not statistically significant.[22]

Untreated acute pyelonephritis is consistently associated with approximately a 30% risk of preterm labor and delivery. Asymptomatic bacteriuria (other than with group B streptococci) has been less consistently associated with an increased risk of LBW.[50-67] Only five of the 17 cohort studies that assessed birth weight reported a significantly increased incidence of prematurity (LBW) in women with asymptomatic bacteriuria.[51, 53, 54, 61, 63] However, in a meta-analysis in which weighting of studies was performed, women with asymptomatic bacteriuria had a 60% higher rate of LBW than nonbacteriuric women (95% confidence interval 1.4 to 1.9).[68] Similarly disagreement exists among the four cohort studies that assessed gestational age as a complication of asymptomatic bacteriuria.[52, 59-61] Two studies demonstrated a significantly increased preterm delivery rate in patients with asymptomatic bacteriuria,[59, 60] while two others did not.[52, 61] In a meta-analysis of all four studies, women with asymptomatic bacteriuria had a 90% higher preterm delivery rate than nonbacteriuric women (95% confidence interval 1.3 to 2.9).[68]

A large number of studies have addressed the issue

Table III. Association of chorioamnion infection* with histologic chorioamnionitis

Series	No.	*Chorioamnion infection*		Odds ratio	95% Confidence interval
		Chorioamnionitis present (%)	*Chorioamnionitis absent (%)*		
Hillier et al.[10]	112	61	23	3.8	1.6-9.9
Pankuch et al.[72]	75	82	15	14	3.6-60
Svensson et al.[73]	87	70	45	2.8	0.6-15.4
Quinn et al.[74]	43	71	28	6.6	1.3-35.3
Zlatnick et al.[75]	95	51	27	3.4	1.2-10.0

*Defined as a positive culture.

of whether maternal genital tract colonization is associated with preterm labor, preterm delivery, or premature rupture of membranes. The results are often conflicting. *N. gonorrhoea* and pyelonephritis have been highly related to preterm birth but their occurrence is infrequent. *U. urealyticum* in the genital tract is not associated with premature delivery. For the remaining lower genital tract microorganisms mentioned and for asymptomatic bacteriuria, the association with prematurity, where found, is low (relative risk 1.4 to 2) and perhaps confined only to subsets. For all of these microorganisms, a more convincing cause-and-effect relationship would exist if the microorganism could be isolated from the amniotic fluid or placenta if women who are delivered of LBW infants have premature rupture of membranes.

Histologic chorioamnionitis and prematurity

Histologic chorioamnionitis, defined as inflammation of the extraplacental membrane, has been consistently linked with prematurity, LBW, and premature rupture of membranes. Histologic chorioamnionitis is detected in 19% to 74% of placentas from preterm deliveries and in 4% to 16% of placentas from term deliveries.[10, 69-71] Although mothers with histologic chorioamnionitis usually have no clinical evidence of infection, their infants have increased rates of sepsis and death.[70] The role of infection in the etiology of histologic chorioamnionitis has been investigated in several recent studies. In both preterm and term placentas, microorganisms have been recovered in 51% to 71% of placentas with 23% to 45% of those without histologic chorioamnionitis (Table III).[10, 72-75] The relationship between histologic chorioamnionitis and infection (positive cultures) of the chorioamnion is strongest among preterm deliveries[10] while it is less strong in term placentas.[76]

In spite of the strong statistical associations between chorioamnionitis and placental membrane infection, 18% to 49% of placentas with histologic evidence of chorioamnionitis are culture negative. This may reflect lack of culture sensitivity or a nonmicrobial cause of inflammation. From 15% to 45% of infected membranes are free of inflammation (Table III). Chorioamnion infection without inflammation may be related either to infection that starts at or near the time of delivery (before an inflammatory response is produced) or to contamination by vaginal bacteria. The infrequency of lactobacilli (a common vaginal contaminant) in placental cultures argues against frequent contamination (Table IV).

Microorganisms most commonly isolated from the chorioamnion include *U. urealyticum*, facultative and anaerobic gram-positive cocci, *G. vaginalis*, and *Bacteroides sp.* (Table IV). Surprisingly, coliforms are uncommon isolates. Likewise, *Haemophilus influenzae* and *N. gonorrhoeae* are rarely recovered from the chorioamnion even though these organisms can cause chorioamnionitis when they are present.[77, 78] In spite of widespread attempts, *C. trachomatis* is rarely recovered from the placenta. Fastidious anaerobes including *Mobiluncus* and *Fusobacterium sp.* may be underrepresented in current studies because of difficulties in isolating these organisms. Thus species of the vagina are found among microorganisms causing placental infection, but many such species appear to have tropism for the placenta.

While the association of histologic chorioamnionitis and prematurity has been well described, the effects of chorioamnion infection on the neonate are less understood. Histologic chorioamnionitis has been associated with an increased perinatal death rate, and infants born to women with chorioamnionitis have had a high prevalence of elevated antibody to *U. urealyticum*.[74] The effects of chorioamnion infection on neonatal outcome require further study to determine which placental pathogens present the greatest risk for poor neonatal outcome.

How does chorioamnion infection lead to premature rupture of membranes and/or preterm delivery? Schoonmaker et al.[79] have demonstrated that group B streptococci significantly decreased placental membrane integrity (i.e., lower bursting tension, less work to rupture, and less elasticity) in vitro, suggesting that placental infection could predispose to membrane rupture. Bernal et al.[80] have demonstrated that the fetal membranes from women with chorioamnionitis produce significantly higher levels of prostaglandin E_2 in vitro. It is likely that production of prostaglandin E_2 is

Table IV. Microorganisms recovered from chorioamnion infection

	Chorioamnion culture (%)					
	Hillier et al.[10] *(n = 112)*	*Pankuch et al.*[72] *(n = 75)*	*Svensson et al.*[73] *(n = 87)*	*Quinn et al.*[74] *(n = 43)*	*Zlatnick et al.*[75] *(n = 95)*	*Overall (N = 412)*
Aerobes						
Group B streptococci	4	5	3	2	5	4
Enterococci	0	5	1	0	1	2
Viridans streptococci	2	0	14	0	4	5
Staphylococci	0	12	16	2	0	6
G. vaginalis	13	0	0	0	4	4
Lactobacilli	1	0	0	0	0	0.2
Coliforms	0	1	2	2	1	0.7
Anaerobes						
Bacteroides sp.	4	7	2	4	4	4
Peptostreptococcus sp.	4	20	6	0	8	8
Genital mycoplasmas						
M. hominis	6	ND	ND	2	4	5
U. urealyticum	29	ND	ND	28	22	26
Yeast	0	1	2	0	0	0.7

ND, Not done.

significantly higher in women with chorioamnion infection. Lamont et al.[81] have demonstrated the *Bacteroides*-conditioned media–stimulated production of prostaglandin E_2 by human amnion cells in vitro. These authors suggested that bacterial phospholipase releases arachidonic acid from the amnion, leading to prostaglandin E_2 synthesis. Whereas further studies of how chorioamnion infection may cause preterm delivery are needed, the published data suggest that chorioamnion infection is strongly related to inflammation and weakening of the placental membrane and to stimulation of prostaglandin production by the amnion.

Amniotic fluid culture in premature labor

Further evidence to link prematurity with infection comes from amniotic fluid cultures of patients with intact membranes in preterm labor. In view of differences in sample size, in definition of preterm labor, and in microbiologic techniques, it is not surprising that the percent of patients with positive cultures varied widely from 0% to 30%[82-91] (Table V). In a recent large series positive cultures were present in 11% of 264 women in preterm labor but in 22% of 111 women who were delivered within 24 to 48 hours of admission.[87] Patients in premature labor and with a positive amniotic fluid culture are usually delivered within 24 to 48 hours in contrast to culture-negative patients who tend to be delivered >30 days later.[87, 89]

These data provide strong circumstantial, but not strong causal, evidence that infection causes the preterm delivery. Difficulties in demonstrating a cause-and-effect relationship would require providing evidence (1) that infection preceded labor rather than vice versa, (2) that cultures are less frequently positive in comparable patients in term labor, and (3) that antibiotic therapy of those infected reduces preterm delivery.

Indirect "markers of infection" in premature labor

Amniotic fluid cultures may be an insensitive test to detect infection that begins in the decidua or amnion. In fact, a high rate of positive chorioamnion cultures occurs in preterm births.[10] Other "markers" of infection that have been assessed in patients in preterm labor include elevated C-reactive protein levels in maternal sera and short-chain organic acids (produced by bacterial metabolism) in amniotic fluid, detected by gas-liquid chromatography. Elevated serum C-reactive protein levels are part of the acute-phase reaction. Most women (86% to 88%) in preterm labor and with high C-reactive protein values do not respond to tocolytics.[92-94] In contrast, most patients with a normal C-reactive protein level responded to tocolytics (77% to 94%).[92-94]

Detection of abnormal organic acids in amniotic fluid was reported in all of six women in preterm labor, but in comparison none of five normal women not in labor had abnormal amniotic fluid acid levels. Only one had a positive culture. The authors suggested that the origin of these abnormal organic acids is bacterial growth in the uterine cavity.[95] The report of Wager et al.[96] did not confirm the value of the gas-liquid chromatography in this setting.

Other biochemical markers are discussed in the next section.

Biochemical mechanisms linking prematurity and infection

Prostaglandins. Until recently, prostaglandins have been considered the universal mediators of parturition in mammalian species.[97-101] Traditional evidence that supports the participation of prostaglandins in the mechanism of labor in humans includes the following: (1) Administration of prostaglandins results in abortion

or labor; (2) treatment with prostaglandins inhibitors delays the process of midtrimester abortion and the onset of labor and can arrest preterm labor; (3) parturition at term is associated with elevated amniotic fluid and maternal plasma concentrations of prostaglandins; (4) arachidonic acid (prostaglandin precursor) concentrations in the amniotic fluid increase during labor; (5) intraamniotic administration of arachidonic acid results in labor.

The evidence supporting a role for prostaglandins in the mechanisms responsible for preterm labor is less firm than for term labor.[101-111] In both plasma and amniotic fluid, levels of prostaglandins have been either normal[100-102] or increased[103, 104] in women in preterm labor. The discrepancies between these studies may be attributed to the heterogeneous nature of diseases causing preterm labor. Patients with preterm labor and microbial invasion of the amniotic cavity have significantly higher amniotic fluid concentrations of prostglandin E_2 and $F_{2\alpha}$ and their stable metabolites (bicycloprostaglandin E_2 and 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$) than women in preterm labor with negative amniotic fluid cultures.[105-111] In contrast, amniotic fluid concentrations of prostaglandins E and $F_{2\alpha}$ are not different between women in preterm labor with negative amniotic fluid cultures and women without labor with negative cultures at similar gestational ages.[108] These findings are consistent with the observation that the production of prostaglandins by amnion and chorion-decidua is higher in patients with preterm labor and histologic chorioamnionitis than in patients with no evidence of placental inflammation. These data suggest an increased bioavailability of prostaglandins in preterm parturition associated with infection. It remains to be proved whether prostaglandins play a role in preterm labor not associated with infection.

Arachidonate lipoxygenase metabolites. Metabolites of arachidonic acid derived through the lipoxygenase pathway involving leukotrienes and hydroxyeicosatetraenoic acids have also been implicated in the mechanisms of spontaneous parturition at term.[112-117] Arachidonate lipoxygenase products are inflammatory mediators, so it is possible that they also participate in the mechanisms of preterm labor associated with infection. Concentrations of 5-hydroxyeicosatetraenoic acid, leukotriene B_4, and 15-hydroxyeicosatetraenoic acid are increased in the amniotic fluid of women with preterm labor and with microbial invasion of the amniotic cavity.[118-120] Similarly, amnion from patients with histologic chorioamnionitis releases more leukotriene B_4 in vitro than amnion from women delivered preterm without inflammation.[121]

The precise role of arachidonate lipoxygenase products in parturition in association with infection remain to be determined. 5-Hydroxyeicosatetraenoic acid and leukotriene C_4 may stimulate uterine contractility, and leukotriene B_4 may recruit neutrophils to the site of infection and participate in the regulation of the cyclooxygenase pathway.[122, 123] Leukotriene B_4 has been shown to act as a calcium ionophore[124] and thus may increase phospholipase activity and enhance the rate of prostaglandin synthesis by intrauterine tissues.

Table V. Amniotic fluid culture results from patients in premature labor

Series and year	*No.*	*Positive (%)*
Harger et al.,[91] 1991	38	0
Weibel and Randall,[82] 1985	35	3
Wahbeh et al.,[83] 1984	27	4
Duff and Kopelman,[84] 1987	24	4
Skoll et al.,[90] 1989	127	6
Leigh et al.,[85] 1986	59	12
Miller et al.,[86] 1980	15	20
Romero et al.,[87] 1989	264	20
Bobitt et al.,[88] 1981	29	21
Gavett et al.,[89] 1986	54	24

Bacterial products. For the past decade the explanation of the onset of labor in the setting of infection has been that bacterial products directly stimulate prostaglandin biosynthesis[125, 126] and bacterially conditioned media stimulate prostaglandin production by human amnion.[127-129] Although the specific product(s) responsible for this effect has not been established, one candidate is endotoxin (lipopolysaccharide). Lipopolysaccharide is a component of the wall of gram-negative bacteria, and it can stimulate prostaglandin production by macrophages, amnion,[130] and decidua.[131] Lipopolysaccharide has been found in the amniotic fluid of women with intraamniotic infections,[132, 133] and in cases of preterm premature rupture of membranes, amniotic fluid concentrations of endotoxin are higher in patients in labor than in patients not in labor.[134] Other bacterial products that may stimulate prostaglandin production are peptidoglycans in cases with gram-positive bacterial infection.

However, bacterial products alone may not be responsible for the onset of preterm labor in the setting of infection. One third of women with preterm premature rupture of membranes without labor have organsims in the amniotic cavity, suggesting that the presence of microorganisms in the amniotic cavity per se is not sufficient to produce labor. Further, the effects of microbial products on prostaglandin production by intrauterine tissues are highly dose dependent and may even be inhibitory.[135] The quantitative aspects of these biologic effects appear important. For example, the concentrations of lipopolysaccharide required to elicit prostaglandin production by amnion cells are rarely present in the amniotic cavity.[134]

Cytokines. The traditional view has been that through the release of toxins or enzymes the microorganisms were directly responsible for the metabolic

derangements associated with infection. It has now been established that endogenous host products secreted in response to infection are responsible for many of the effects of infection. In endotoxin shock, for example, bacterial endotoxins exert their deleterious effect through the release of endogenous mediators such as tumor necrosis factor and interleukin-1.

Parturition in the setting of infection may be signaled by secretory products of macrophage activation (monokines), including interleukin-1, tumor necrosis factor, and interleukin-6. The first monokine implicated in the onset of labor in the setting of infection was interleukin-1: (1) Interleukin-1 stimulates prostaglandin production by amnion and decidua in vitro[136]; (2) human decidua can produce interleukin-1 in vitro in response to bacterial products[137, 138]; (3) amniotic fluid interleukin-1 bioactivity and concentrations are elevated in patients with preterm labor and bacteria in the amniotic cavity,[139] but interleukin-1 is not present in fluid from patients with preterm labor and no bacteria; (4) among patients with preterm premature rupture of membranes and bacteria in the amniotic cavity, amniotic fluid interleukin-1 bioactivity and concentrations are higher in patients with than in those without labor,[139] indicating it is not the presence of microorganisms per se but rather the host response that is associated with parturition; (5) in vitro perfusion of human uteri with interleukin-1 elicits uterine contractility (unpublished data); (6) administration of interleukin-1 to pregnant animals leads to abortion and preterm labor (Romero R, Mazor M, Tartakovsky B. Unpublished observations).

Tumor necrosis factor, a cytokine released during macrophage activation, is also capable of stimulating prostaglandin production by several cell types. Evidence supporting a role of tumor necrosis factor in human parturition in the setting of infection includes: (1) Tumor necrosis factor stimulates prostaglandin production in vitro by human amnion and decidua[131, 140]; (2) tumor necrosis factor is produced by human decidua in response to bacterial products[141, 142]; (3) tumor necrosis factor is absent in normal amniotic fluid but present in the amniotic fluid of patients with intraamniotic infection and preterm labor[140]; (4) tumor necrosis factor can induce premature labor when administered to pregnant mice (Romero R. Unpublished observations).

Interleukin-6 is also a major mediator of the host response to infection. Interleukin-6 is produced by a variety of cells including macrophages and endometrial stromal cells. Major change in the biochemical, physiologic, and immunologic status of the host occurs from interleukin-6, including an acute-phase plasma protein response and the induction of other cytokines such as interleukin-1 and tumor necrosis factor. Women in preterm labor with intraamniotic infection had higher amniotic fluid levels of interleukin-6 than women in preterm labor without intraamniotic infection.[143, 144]

Macrophage colony-stimulating factor is a cytokine that is capable of regulating the number of macrophages and their level of activation. Macrophage-colony stimulating factor is produced by human decidual explants in response to lipopolysaccharide and it is present in the amniotic fluid of women in preterm labor with intraamniotic infection.

Noncytokine bioactive agents secreted during the inflammatory process may also participate in this process. Platelet-activating factor is a lipid that is present in the amniotic fluid of women with preterm labor. This compound is capable of stimulating prostaglandin E_2 production by amnion and of directly stimulating myometrial contractions.

This evidence suggests that the host products of macrophage activation (cytokines) play a role in the mechanism of human parturition in the setting of infection. Systemic maternal infections, such as pyelonephritis, or localized infections, such as deciduitis, could signal parturition through monocyte-macrophage activation in peripheral blood and decidua. Preterm labor can be viewed as an event occurring when the intrauterine or maternal environment is hostile and threatens the well-being of the fetus. The initiation of preterm labor in the setting of infection may be considered to have survival value.

Antibiotic trials to prevent premature birth

With the evidence that subclinical infection is a major cause of preterm birth, the logical extension is to conduct antibiotic intervention trials in an attempt to prevent prematurity. Trials to date have generally been of three designs: (1) antibiotic trials conducted antepartum in patients at high risk for preterm delivery; (2) antibiotic trials among women with premature labor but with intact membranes, as adjuncts to standard tocolytic therapy; and (3) antibiotic trials among women with preterm premature rupture of membranes but without preterm labor.

In the first antepartum antibiotic trial, patients with genital mycoplasmas in the lower genital tract were enrolled in a trial of erythromycin versus placebo for 6 weeks.[30, 145] Women in the erythromycin group, as compared with the placebo group, had a significantly higher mean birth weight (3331 vs 3187 gm, $p = 0.04$) and a lower rate of LBW (3% vs 12%, $p = 0.06$). In a study sponsored by the National Institutes of Health conducted at five centers, >900 patients with lower genital tract *U. urealyticum* were randomized to either erythromycin or placebo from the twenty-eighth through the thirty-fifth week of gestation.[42] Patients in the erythromycin group, compared with the placebo group, ex-

Table VI. Summary of antibiotic trials to delay delivery in preterm labor

Series and year	*No.*	*Regimen*	*Significant results in antibiotic group*			
			Delay in delivery	*Increase in birth weight*	*Increase in term delivery*	*Decrease in perinatal mortality*
McGregor et al.,[149] 1986	17	Erythromycin orally for 7 days	Yes	$p = 0.07$	No	No
Newton et al.,[150] 1989	95	Ampicillin intravenously for 2 days plus erythromycin for 4 days	No	No	No	No
Morales et al.,[151] 1988	150	Ampicillin orally or erythromycin orally, either for 10 days	Yes	No	Yes*	No
Winkler et al.,[152] 1988	19	Erythromycin orally for 7 days	Yes	No	Not reported	No

*For ampicillin versus placebo.

perienced no improvement in any of the following measures: rate of LBW, premature labor, preterm delivery, or preterm premature rupture of membranes. In the third study 229 patients at high risk for preterm delivery were randomized to a 7-day course of erythromycin or placebo.[146] No significant differences were present between the erythromycin and the placebo groups in preterm birth (7% vs 8%), mean birth weight (3132 ± 588 vs 3099 ± 548 gm), or preterm premature rupture of membranes (1.7% vs 4.6%). A significant reduction was observed in premature rupture of membranes at term between the groups (6% vs 16%, $p = 0.01$).

In two studies patients with cervical *C. trachomatis* were assessed for the effect of antibiotic treatment of pregnancy outcome. Patients successfully treated for *C. trachomatis* ($n = 244$) had significantly lower rates of premature rupture of membranes, premature labor, and small-for-gestational-age infants when compared with those who failed to respond ($n = 79$).[147] In a second report pregnancy outcome was studied in *C. trachomatis*–positive untreated patients ($n = 1110$), *C. trachomatis*–positive treated patients ($n = 1327$), and *C. trachomatis*–negative women ($n = 9111$). The untreated *C. trachomatis*–positive group had significantly increased rates of premature rupture of membranes and LBW and lower survival rates compared with those of both the treated *C. trachomatis*–positive group and those with negative cultures.[148] However, retrospective nonrandomized studies such as these are less convincing because of possible confounding factors.

In the first trial of the adjunctive use of antibiotics in patients in premature labor with intact membranes, a subgroup of patients with cervical dilatation between 1 and 5 cm who received adjunctive erythyromycin, as compared with the placebo group had significant prolongation of pregnancy (32 ± 11 vs 22 ± 7 days, $p = 0.02$), a significant improvement in patients delivered at term (87% vs 33%, $p = 0.04$), and an improvement in birth weight (2943 ± 483 vs 2615 ± 459 gm, $p = 0.07$).[149] Three additional studies of similar design have been reported.[150-152] In patients in preterm labor who received either ampicillin plus erythromycin for 4 days or placebo, there was no decrease in preterm birth (38% in the antibiotic group vs 44% in the placebo group).[150] In patients in idiopathic preterm labor randomized to one of the following regimens: ampicillin orally for 10 days, erythromycin orally for 10 days, or no antibiotic,[151] those receiving either ampicillin or erythromycin had significant prolongation of the pregnancy (31.7 days for ampicillin vs 28.5 days for erythromycin and 16.6 days for controls, $p < 0.05$) and patients receiving ampicillin compared with those receiving placebo also had a significantly higher rate of term delivery (45% vs 15%, $p < 0.05$). Among patients in idiopathic preterm labor randomized to either erythromycin for 7 days or placebo, a significant prolongation of the pregnancy occurred in the erythromycin group versus the placebo group (43 ± 25 vs 10 ± 19 days, $p < 0.05$).[152] Although there may be several reasons to explain the inconsistency of antibiotics in delaying delivery, there appear to be differences regarding the investigators' "intent to treat." For example, in the only "negative study" a high percentage of enrolled women were analyzed[150] whereas in the "positive studies" the percentage of enrolled patients ultimately analyzed was small.[149, 151] Even when antibiotic treatment has led to a prolongation of pregnancy, its use has not been accompanied by a consistent increase in birth weight and has not led to a decrease in perinatal mortality (Table VI).

Several recent randomized antibiotic trials have been conducted among patients with preterm premature rupture of membranes (Table VII). The hypothesis of these studies was that subclinical infection either causes premature rupture of membranes as a primary event

Table VII. Summary of antibiotic trials to delay delivery after preterm premature rupture of membranes

Series and year	No.	Regimen	Significant results in antibiotic group			
			Delay in delivery	*Increase in birth weight*	*Increase in term delivery*	*Decrease in perinatal mortality*
Amon et al.,[153] 1988	83	Ampicillin intravenously, then orally until delivery	Yes	No	No	No
Morales et al.,[154] 1989	82	Ampicillin intravenously "pending cultures"	Yes	No	No	No
Johnston et al.,[155] 1990	85	Mezlocillin intravenously, ampicillin orally until delivery	Yes	Yes	Yes	No
Christmas et al.,[156] 1990	56	Ampicillin gentamicin, clindamyin intravenously for 1 day, amoxicillin and clavulanic acid for 7 days	Yes	No	No	No

or causes the premature labor that ensues after preterm premature rupture of membranes. A significant increase was observed in pregnancies delivered >1 week after premature rupture of membranes in the ampicillin group versus the no-ampicillin group (47% vs 26%, $p = 0.05$).[153] This prolongation of pregnancy was not accompanied by a significant difference between groups in birth weight, gestational age at delivery, or hospital stay >30 days. In another report patients with preterm premature rupture of membranes were given betamethasone and randomized to ampicillin or no ampicillin.[154] No significant difference was found between those who had and those who had not received ampicillin in birth weight or in survival (93% vs 89%). Patients receiving ampicillin compared with those not receiving ampicillin had significantly less clinical amnionitis (4% vs 26%, $p < 0.01$). In the third study neither corticosteroids nor tocolytics were given to patients with preterm premature rupture of membranes.[155] Patients randomized to antibiotics versus placebo had a significant prolongation of pregnancy ≥7 days (45% vs 18%, $p < 0.01$), a significant increase in birth weight (1897 ± 600 vs 1587 ± 592 gm, $p < 0.05$), and a decrease in neonatal hospital stay >30 days (18% vs 38, $p < 0.05$). No significant differences were reported in perinatal mortality. In a smaller study antibiotic therapy compared with expectant management of patients with preterm premature rupture of membranes was associated with prolongation of pregnancy ≥8 days (36% of antibiotic groups vs 10% of expectant group, $p < 0.05$) but with no significant difference in mean birth weight, perinatal mortality, or respiratory distress syndrome.[156] These authors concluded that intervention with antibiotics delayed labor but offered no appreciable perinatal advantage.

In addition to these randomized trials there have been several interesting, innovative reports regarding the use of antibiotics in premature rupture of membranes. Successful eradication of "bacterial colonization" of the amniotic fluid was achieved by parenteral maternal antibiotic therapy in a preterm gestation with premature rupture of the membranes at 29 weeks' gestation.[57] Transcervical infusion of antibiotics into the amniotic cavity has occurred in patients with preterm premature rupture of membranes.[158, 159] Substantial antibiotic concentrations were achieved in the amniotic fluid, but the safety and efficacy of this approach have not been established.

In conclusion, current antibiotic trials allow no definite conclusion as the efficacy of antibiotics in prolonging pregnancy in patients with either preterm labor or preterm premature rupture of membranes. These discordant results may result from population differences. Antibiotics would be expected to have no effect, for example, among the subset of women with preterm labor–premature rupture of membranes if the cause is not infection. Differences in analysis of eligible patients also may have been important. For example, a positive study might be more likely if the investigators limited analysis to patients who completed versus those who did not complete the antibiotic course. Additional trials seem appropriate in view of results to date. However, randomized trials need to be conducted in selected groups at high risk for preterm delivery and/or infection or preferably in groups with specific infections or with evidence of preexisting infection such as histologic chorioamnionitis.

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In the rhesus monkey placental retention after fetectomy at 121 to 130 days' gestation outlasts the normal duration of pregnancy

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OBJECTIVE: Our aim was to reassess the role of the fetus in the initiation of parturition in nonhuman primates. We tested the effect of the removal of the fetus at 121 to 130 days' gestation on the duration of gestation in pregnant rhesus monkeys.
STUDY DESIGN: Nine monkeys underwent fetectomy with the placenta in situ. Five monkeys underwent surgery without removal of the fetus.
RESULTS: In five control monkeys spontaneous vaginal delivery of live fetuses occurred at 163.8 ± 4.6 days' gestation (mean ± SD). In four of nine monkeys that underwent fetectomy the placenta delivered spontaneously at 185, 193, 201, and 207 days' gestation. The five remaining monkeys underwent cesarean section at 162, 189, 201, 202, and 219 days' gestation. Duration of placental retention in monkeys that underwent fetectomy (195 ± 16.1 days' gestation) exceeded that in controls ($p < 0.05$). Plasma progesterone and estradiol concentrations were normal for gestational age at fetectomy, indicating continued placental function.
CONCLUSION: We conclude that the presence of a live fetus plays a significant role in the determination of the duration of normal pregnancy in the rhesus monkey. (Am J Obstet Gynecol 1992;166:1529-35.)

Key words: Parturition, fetectomy, estradiol, progesterone, placenta, gestation length

The fetus plays a major role in the determination of the duration of gestation in the sheep. In this species decreased function of the fetal hypothalamopituitary-adrenal axis after placement of bilateral fetal paraventricular lesions,[1] fetal pituitary stalk section, fetal hypophysectomy, and bilateral fetal adrenalectomy[2] results in the prolongation of gestation. Conversely, infusions of either adrenocorticotropic hormone[2] or physiologic doses of cortisol[3] intravascularly to the fetal sheep will initiate parturition prematurely. It is unclear whether the primate fetus actively participates in the initiation of parturition. Controversy persists partly on the basis of results obtained in two studies that used different experimental approaches. First, large doses of glucocorticoids administered to the pregnant rhesus monkey will prolong gestation.[4] Second, in one often-quoted study removal of the fetus from 11 pregnant monkeys at a wide range of gestational ages (70 to 157 days' gestation) resulted in delivery of the placenta between 114 and 184 days' gestation. These authors stated, "The onset of labor is shown to be unquestionably independent of the immediate presence of the fetus."[5]

Somewhat different results were obtained by Lanman et al.,[6] who performed fetectomy in 13 pregnant rhesus monkeys. In this study the fetuses were also removed over a wide period, between 60 and 140 days' gestation. Estradiol or dexamethasone was administered to five of the 13 monkeys in connection with other studies. No contemporaneous control animals were used in either the study of Lanman et al. or the study by van Wagenen and Newton.[5] Lanman et al.[6] stated that in five of the eight animals that underwent fetectomy but received no other treatment the placenta was delivered outside the normal gestational age limits. Two of these five animals were only 4 days outside the given limits when the placenta was delivered.

To evaluate the role of the nonhuman primate fetus in determining the duration of gestation, instrumentation and fetectomy were performed in nine pregnant rhesus monkeys and five control pregnant monkeys underwent instrumentation in a similar way without fetectomy. Our major goal was to obtain data that would allow firm statistical comparison between the duration of placental retention after fetectomy and the duration of pregnancy in contemporaneous sham-operated controls. We also wished to evaluate the maintenance of plasma progesterone and estradiol concentrations after fetectomy. In addition, we wished to compare the pat-

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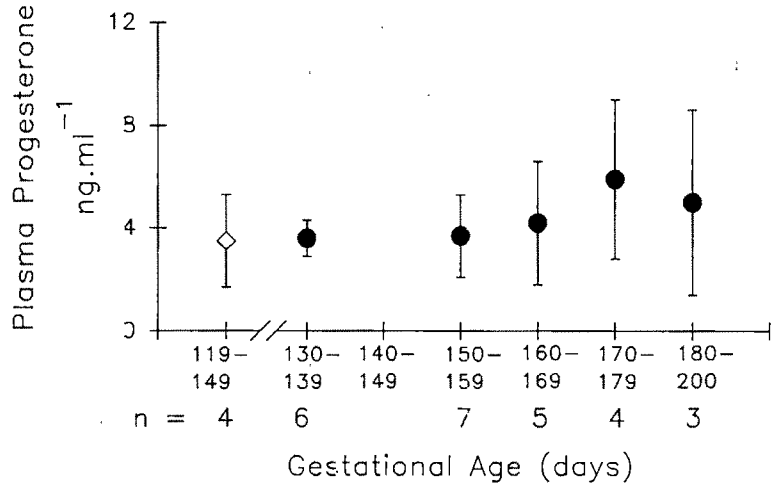


Fig. 1. Plasma progesterone concentrations in rhesus monkeys after fetectomy (●) at different gestational age windows (mean ± SD). Plasma progesterone in four control monkeys is shown (◇) as mean ± SD at 119 to 149 days' gestation. *n*, Number of animals sampled at each gestational age.

terns of myometrial activity observed in pregnant monkeys after fetectomy with previous observations in intact pregnant rhesus monkeys at similar stages of gestation.[7]

Material and methods

Care of animals. Fourteen pregnant rhesus monkeys of known gestational age from the California Regional Primate Research Center (Davis, Calif.) were studied. They were acclimated to the laboratory conditions as previously described.[8] Photoperiod in the animal laboratory was 14 hours light and 10 hours dark with lights off at 2 PM. Instrumentation was performed in five control pregnant rhesus monkeys at 99 to 107 days' gestation with a maternal femoral artery and vein catheter and myometrial electromyogram leads. An intrauterine pressure catheter was placed in the uterus through a small incision, as previously described.[9] In a further nine pregnant monkeys surgery was conducted at 121 to 130 days' gestation. In addition to the placement of the above-mentioned instrumentation, the uterine incision was extended to permit removal of the fetus. The fetus was euthanized by exsanguination while it was still under the influence of halothane general anesthesia. The umbilical cord was ligated twice, followed by section between the ties and removal of the fetus, with the placenta left in situ.

The monkeys were maintained postoperatively on a tether-and-swivel system that permitted sampling of maternal blood and recording of myometrial activity.[9] Blood samples for measurement of plasma progesterone levels were taken at three fixed times in relation to the lighting regimen: 4 and 9 hours after the lights went on and 1 hour after the lights went off. The mean value for each day was used for statistical analysis. Plasma estradiol value for each individual animal was the mean of 15 samples taken at 4-hour intervals for 56 hours. Cesarean section was performed with animals under halothane general anesthesia to remove the placenta and terminate the study in five of the monkeys that underwent fetectomy.

Radioimmunoassay procedures. The radioimmunoassays used to measure maternal plasma progesterone and estradiol levels in rhesus monkeys have been described in detail.[10]

Recording and analysis of myometrial electromyogram. Myometrial electromyogram signals were recorded on a computer-based data acquisition system and analyzed both as discrete epochs and also by power spectral analysis for dominant specific sine components as previously described.[7]

Statistical analysis. Results are given throughout as mean ± SD, and the two groups were compared by the two-sample *t* test. Significance was accepted at $p < 0.05$.

Results

Gestational age at the time of delivery of the placenta. In the five control monkeys live fetuses were delivered spontaneously at 165, 159, 159, 167, and 169 (163.8 ± 4.6) days' gestation. In four of the nine monkeys that underwent fetectomy the placenta was delivered vaginally at 185, 193, 201, and 207 days' gestation, after the spontaneous onset of uterine contractions. The placenta was removed by cesarean section at 162, 189, 201, 202, and 219 days' gestation in the other five monkeys that underwent fetectomy. The cesarean section at 162 days was necessary because of prolapse of the stump of the umbilical cord through the cervix,

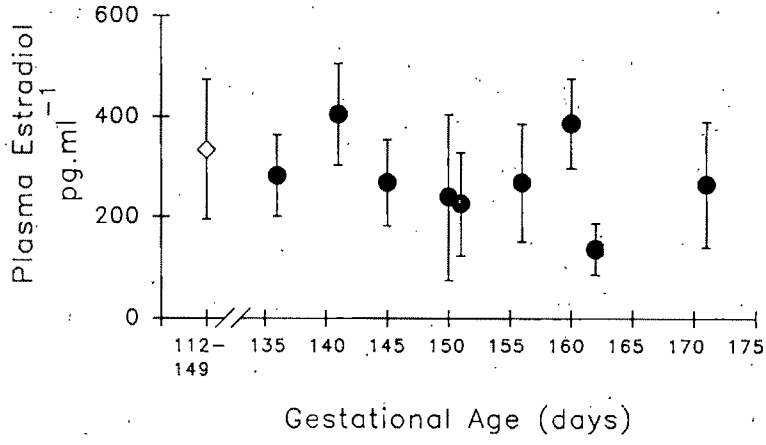


Fig. 2. Plasma estrogen concentration in seven monkeys after fetectomy (●). Each value is mean ± SD of 15 samples taken at 4-hour intervals during 56 hours. For comparison, mean ± SD value for four controls is also shown (◇), with samples over range of 112 to 149 days' gestation.

thereby presenting a risk of intrauterine infection. This animal was not in labor before prolapse. None of the other four monkeys that underwent fetectomy was in labor at the time of elective cesarean section. The gestational age at delivery of the placenta in the group that underwent fetectomy was 195 ± 16.1 days' gestation, which significantly outlasted the duration of pregnancy in the controls ($p < 0.05$).

Maternal arterial plasma progesterone and estradiol concentrations. Plasma progesterone concentrations were well maintained after fetectomy (Fig. 1). At 160 to 169 days' gestation in five animals from which samples were available for progesterone measurement, the plasma progesterone level was 4.2 ± 2.4 ng · ml^{-1}. Maternal plasma estrogen was also well maintained after fetectomy. In the nine animals that underwent fetectomy maternal plasma estradiol (mean over 56 hours) ranged from 282 pg · ml^{-1} at 136 days' gestation to 265.8 pg · ml^{-1} at 171 days (Fig. 2). These values were similar to those found in four control intact rhesus monkeys studied over 112 to 149 days' gestation, 334.6 ± 140 pg · ml^{-1}.

Myometrial activity. In the current study the predominant form of myometrial activity observed before delivery in both groups of monkeys was of the contracture type, which was similar to the activity we observed in >50 intact pregnant rhesus monkeys studied at this stage of gestation and the five controls used in this study. Data from three monkeys that underwent fetectomy and three control monkeys recorded when myometrial activity was in the contracture mode are shown in Fig. 3.

Monkeys that underwent fetectomy did occasionally have contraction-type activity. When this occurred, it happened characteristically during the hours of darkness (Fig. 4) and was clearly distinct from the contracture pattern. In our previous study in pregnant monkeys during contracture activity, power spectral analysis showed the majority of the spectral power (64.7%) below 0.11 cycles · min^{-1}. We designated frequencies <0.11 cycles · min^{-1} as the contracture window. Power spectral analysis was performed on myometrial activity during the night at 30 and 50 days after fetectomy. In eight of the nine animals that underwent fetectomy for which myometrial electromyographic data were available, the myometrial activity present in the contracture window was 61.8% ± 12.9% and 68.1% ± 13.3% at 30 and 50 days after fetectomy, respectively.

We have demonstrated previously that when intact rhesus monkeys go into labor at term there is a switch from contractures to contractions around the time the lights go off in the animal's environment. After a few hours the contractions switch back to contractures. In intact animals the switch from contractures to contractions recurs and intensifies in a 24-hour periodic fashion until delivery eventually occurs (Fig. 5). The switch from contractures to contractions did not show this recurring pattern in fetectomized monkeys in which the placenta was delivered vaginally (Fig. 6).

Placental weight. The placenta weighed 112.8 ± 29.1 gm in the five animals from which it was removed by cesarean section. In the other four animals the placenta was at least partially eaten by the mother and hence the weights were not available. The five fetuses in these pregnancies weighed 338 ± 31.9 gm when removed at fetectomy. According to the data provided by Cheek[11] placental weight can be calculated in the rhesus monkey from the formula: Placental weight = 0.19 (Fetal weight) ± 37.06. With this formula the estimated placental weight at the time the

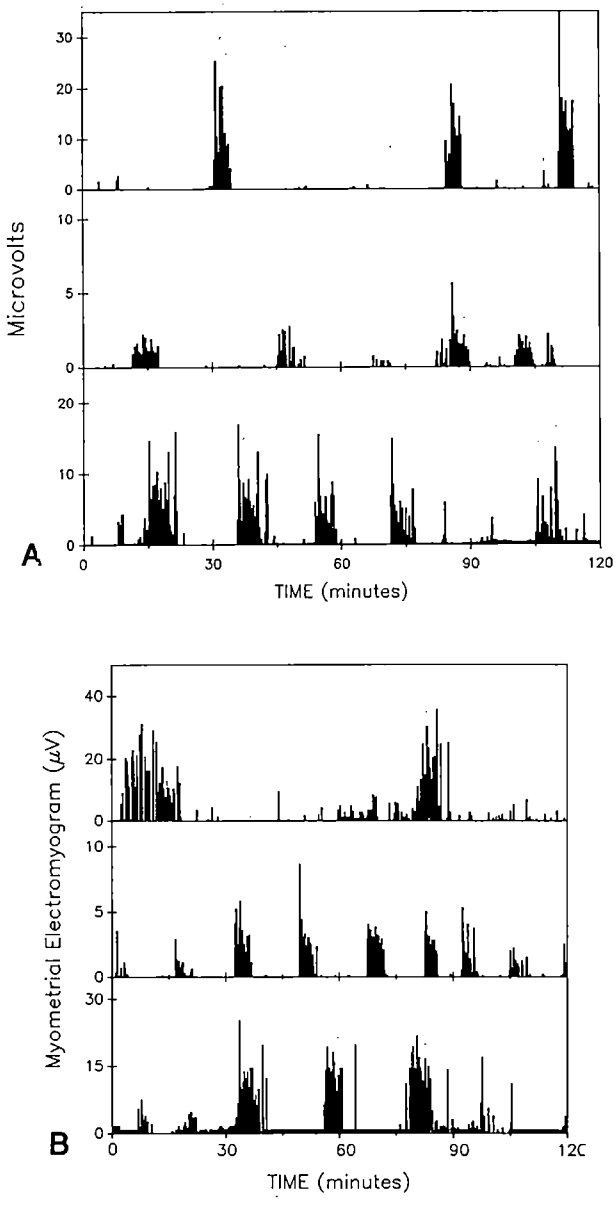


Fig. 3. Myometrial electromyogram recordings to show basic contracture pattern of uterine contractility in three fetectomized monkeys on day 20 after fetectomy **(A)** and three control monkeys **(B)** observed between 141 and 160 days' gestation. Contracture activity was basic form of myometrial activity throughout this period.

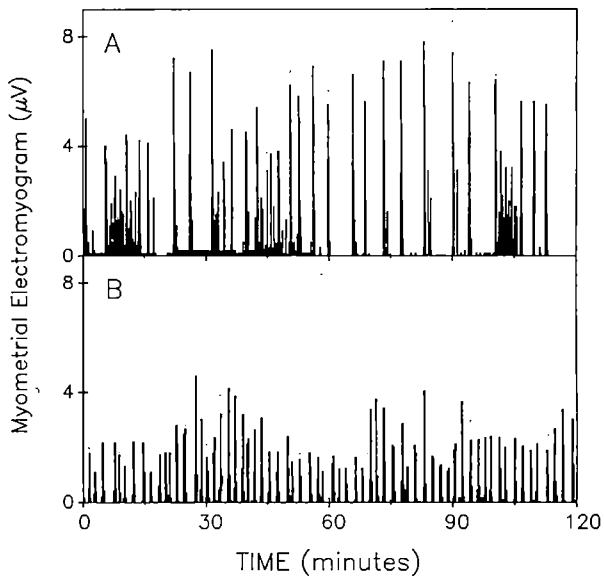


Fig. 4. Similarity of myometrial contraction-type activity as shown by myometrial electromyogram when uterus was in contractions mode in one fetectomized monkey 50 days after fetectomy **(A)** and one control monkey **(B)**.

fetuses were removed would be 101.3 gm. Thus the placentas had remained at about the size they were when the fetus was removed. Placental weights were not available for control monkeys because the mother eats the placenta immediately after vaginal delivery.

Comment

Experiments that clearly define the role of the fetal hypothalamohypophyseal-adrenal axis in the initiation of parturition in the sheep have been difficult to reproduce in nonhuman primates. The nonhuman primate species used for these studies has almost exclusively been the rhesus monkey.[12-14] A major problem in studies that involve intrauterine surgery and fetal instrumentation in this species is the tendency of the monkey to subsequent premature delivery. Therefore comparison of the duration of gestation in pregnant monkeys that have undergone experimental intrauterine surgery on the fetus with the duration of gestation in animals not operated on is less than optimal. This high incidence of premature delivery complicates the evaluation of studies designed to prolong gestation. In the current study we have assessed the fetal role in the determination of the duration of gestation by examining the effect of fetectomy on retention of the placenta and patterns of myometrial activity.

Van Wagenen and Newton[5] performed fetectomy at a very wide gestational age range (70 to 157 days) to investigate the role of the fetus in the initiation of delivery in the pregnant rhesus monkey. In the current study we sought to keep the gestational age at fetectomy within a narrow window (121 to 130 days; approximately 75% of the normal duration of pregnancy, which is 168 days in this species). We chose the window 121 to 130 days' gestation to perform the fetectomy because this period immediately precedes the marked increase in fetal adrenal growth and rise in fetal plasma dehydroepiandrosterone level that precedes birth in the monkey.[15]

When fetectomy data in the available studies in the monkey and other species are compared, it is necessary to take into account the gestational age at which the

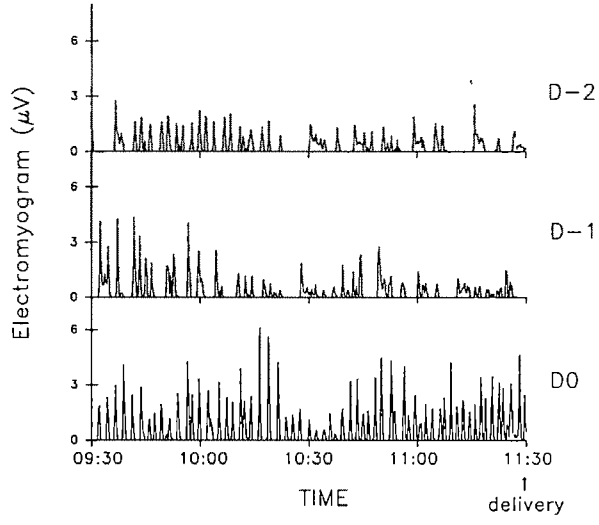


Fig. 5. Myometrial electromyogram from 9:30 to 11:30 PM on day of delivery and two previous nights in intact pregnant rhesus monkey delivered at 165 days' gestation. Lights on at 12 midnight and off at 2 PM. *D-0,* Day of delivery; *D-1* and *D-2,* 1 and 2 days before delivery, respectively.

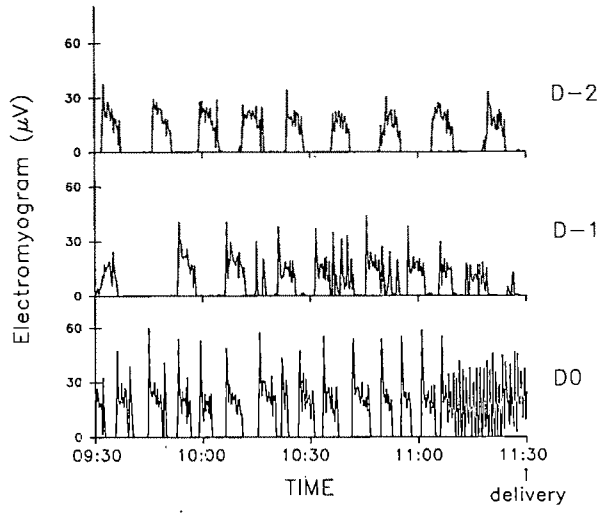


Fig. 6. Myometrial electromyogram from 9:30 to 11:30 PM on day of delivery and two previous nights in monkey after fetectomy that was delivered at 185 days' gestation. Lights on at 12 PM and off at 2 PM. *D-0,* Day of delivery; *D-1* and *D-2,* 1 and 2 days before delivery, respectively.

fetus was removed. In the Van Wagenen and Newton study, in general, the earlier in gestation the fetectomy was performed, the earlier the delivery of the placenta occurred. In another study three monkeys underwent fetectomy at 69 to 73 days' gestation. Fetectomy was followed by spontaneous vaginal delivery of the placenta at 116, 159, and 163 days' gestation.[16] In addition, four monkeys that underwent fetectomy at 107 to 119 days' gestation had the placenta removed by cesarean section at 167, 167, and 169 days, corresponding to the time of normal spontaneous delivery in the rhesus monkey. Thus information on when these placentas could have delivered spontaneously is lacking.[16]

In our studies the placenta remained viable as demonstrated by maternal plasma progesterone (2 to 5 ng · ml^{-1}) and estradiol (250 to 500 pg · ml^{-1}) concentrations that were within the normal midpregnancy range for intact animals in our laboratory and that of others.[17] The weights of the five placentas we were able to measure were maintained at the level they would have been at the time of fetectomy.[11] Normal maternal progesterone concentrations have been observed in all fetectomy studies to date in the rhesus monkey.[16] Tullner and Hodgen[16] demonstrated a fall in maternal estradiol level in one pregnant rhesus monkey that underwent fetectomy at 73 days' gestation and one at 116 days' gestation. In contrast, Dorfman and van Wagenen[18] found no significant change in urinary estrogen excretion after fetectomy.

It is important to compare our findings on placental retention and estrogen and progesterone plasma concentrations in the rhesus monkey after fetectomy with observations made by Albrecht et al.[19-22] in another nonhuman primate species, the baboon, after fetectomy. Both similarities and differences exist. Studies in both species agree that the placenta remains viable after fetectomy[19-22] as determined by the ability of the placenta to convert androgens to estrogen.[19] However, after fetectomy in the baboon at midgestation, maternal estradiol concentrations fall to low or undetectable levels. This observation suggests that in the absence of the fetus the supply of C19 precursors to the placenta is inadequate. The current studies in rhesus monkeys that underwent fetectomy at 75% of gestation show that adequate maternal precursors are available to maintain estrogen concentrations at the level present at the time of fetectomy. The most likely source of androgenic precursors for estrogen production in the monkey after fetectomy is the maternal adrenal. The failure of maternal estrogens to rise after 155 days' gestation supports the view that the fetus provides the precursors for the normal prepartum increase in maternal estrogens.

In the pregnant baboon maternal progesterone falls 64% to 80% after fetectomy.[20] In contrast, maternal progesterone levels were maintained within the normal pregnancy range in the rhesus monkey after fetectomy in both our study and that of others.[16] After fetal death in pregnant women, the maternal progesterone level is maintained although maternal estrogen production decreases.[23] Whether the continued production of progesterone in the rhesus monkey is due to a rejuvenated corpus luteum or maintained placental function remains to be determined. In any event our study dem-

onstrates that in the rhesus monkey, after fetectomy but with retention of the placenta, plasma progesterone and estrogen concentrations are maintained well above nonpregnant levels and within the midpregnancy range. In the baboon after fetectomy in midpregnancy the placenta is delivered at term. Administration of estrogen to the baboon after fetectomy will maintain maternal progesterone concentrations and results in the retention of the placenta in utero past the normal length of gestation.[21, 22] Thus the differences in progesterone production after fetectomy in the monkey and baboon may be determined in large measure by differences in estrogen production. These interesting differences after fetectomy between the baboon and the monkey merit further investigation.

None of the previous studies involving fetectomy have been conducted in chronically instrumented animals to permit continuous evaluation of myometrial activity. In the current study we observed that myometrial activity after fetectomy in the monkey throughout the period of observation consisted almost entirely of long-lasting, contracture-type myometrial epochs. This type of myometrial activity is the predominant form of myometrial contractility that may be observed during pregnancy in the rhesus monkey[7] and baboon.[24] In monkeys after fetectomy contractures switch to contractions when the placenta is delivered. As with intact pregnant rhesus monkeys, this switch occurs around the time the lights go off in the animal's environment. However, the frequency of recurrence of the shift from contracture to contraction patterns is less and its amplitude less pronounced when monkeys that have undergone fetectomy are delivered, compared with intact monkeys.

In summary, after fetectomy in the pregnant rhesus monkey at 120 to 130 days' gestation, the placenta may be retained as long as 45 days after the average duration of pregnancy in controls. These results support the concept that the fetus plays a role in determining the length of gestation in this nonhuman primate species.

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The effect of benzo(a)pyrene on murine ovarian and corpora lutea volumes

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OBJECTIVE: Women who smoke have impaired fertility and experience menopause at an earlier age. This experiment determined the effect of benzo(a)pyrene, a polycyclic aromatic hydrocarbon contained in cigarette smoke, on murine ovarian volume, total corpora lutea volume, individual corpora lutea volumes, and corpora lutea numbers.

STUDY DESIGN: C57BL/6N mice were treated with intraperitoneal injections of 0 to 500 mg/kg benzo(a)pyrene in corn oil. The 20 mice at each dose were divided into four groups of five each and were killed at 1, 2, 3, or 4 weeks after treatment. Ovaries were serially sectioned and analyzed morphometrically.

RESULTS: Benzo(a)pyrene produced a dose- and time-dependent decrease in ovarian volume, total corpora lutea volume, and number of corpora lutea per ovary. This effect was transitory at low doses with complete recovery of corpora lutea by 4 weeks. Compensatory hypertrophy of the individual corpora lutea occurred during the recovery phase. Ovarian function did not return in animals treated with the two highest doses.

CONCLUSION: Benzo(a)pyrene is a murine ovarian toxicant that inhibits corpus luteum formation in a dose- and time-dependent fashion. (AM J OBSTET GYNECOL 1992;166:1535-41.)

Key words: Corpora lutea, ovary, smoking, polycyclic aromatic hydrocarbons

There is a growing weight of evidence that suggests that women who smoke have impaired fecundity, an increased risk for infertility, and a younger age at menopause. Available data exploring these adverse reproductive effects with epidemiologic techniques suggest that many, if not all, of these adverse outcomes are cigarette dose related. Recently these adverse reproductive effects of smoking have been thoroughly reviewed.[1]

Cigarette smoke is a highly complex mixture containing approximately 4000 identified compounds.[2] Those that have attracted the greatest attention produce toxic effects in organ systems other than the reproductive tract. These compounds include carbon monoxide, nicotine, and chemicals contained in the tar fraction, including polycyclic aromatic hydrocarbons. Previous investigations in this and other laboratories have explored the effect of individual components of cigarette smoke on reproductive function in experi-

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Table I. Total ovarian volume (in cubic millimeters, mean ± SD)

Dose (mg/kg)	*Week 1**	*Week 2**	*Week 3**	*Week 4**
Control	0.91 ± 0.08	0.90 ± 0.18	0.82 ± 0.25	0.82 ± 0.10
1	0.76 ± 0.27	0.70 ± 0.16†	0.78 ± 0.21	0.64 ± 0.21
5	0.61 ± 0.12‡	0.89 ± 0.16	0.87 ± 0.16	0.75 ± 0.21
10	0.64 ± 0.10‡	0.85 ± 0.22	0.73 ± 0.23	0.85 ± 0.17
50	0.50 ± 0.14‡	0.68 ± 0.17§	0.63 ± 0.29	0.67 ± 0.22
100	0.50 ± 0.22‡	0.47 ± 0.11‡	0.57 ± 0.21†	0.43 ± 0.21‡
500	0.32 ± 0.09‡	0.13 ± 0.10‡	0.13 ± 0.04‡	0.13 ± 0.02‡

*$p < 10^{-5}$.
†$p < 0.05$.
‡$p < 0.0001$.
§$p < 0.01$.

mental animals.[1] Our attention has focused on the impact of polycyclic aromatic hydrocarbons on ovarian function; we have demonstrated that some of these hydrocarbons can destroy follicles and impair fertility in mice.[1, 3, 4] Recent investigations have suggested that the impact of polycyclic aromatic hydrocarbons on impaired fertility is a result of an alteration in ovulatory frequency.[4] To further investigate this impact on ovulatory frequency, we have conducted a series of studies designed to characterize the effect of benzo(a)pyrene, a common environmental polycyclic aromatic hydrocarbon and major constituent in cigarette smoke, on total ovarian volume, total corpora luteal volume, the number of corpora lutea per ovary, and the volumes of individual corpora lutea at 1, 2, 3, and 4 weeks after treatment with varying doses.

Material and methods

A total of 140 weanling C57BL/6N mice were obtained from the National Institutes of Health veterinary resources branch. Animals were maintained in the laboratory for approximately 1 week to allow acclimation. Care and use of animals conformed to the guidelines established by the animal care committee. Animals were divided into groups of 20 and injected with one of seven doses of benzo(a)pyrene, 0, 1, 5, 10, 50, 100 or 500 mg/kg. Doses were given in corn oil intraperitoneally, such that a 20 gm mouse received 1 ml corn oil. Each dosage group was then subdivided into groups of five. These subgroups of animals were killed at 1, 2, 3, or 4 weeks after treatment. Then the ovaries were removed and fixed for 24 hours in Bouin's fixative. They were then placed in 70% ethanol until embedding, serially sectioned at 5 μm, and stained with hematoxylin and eosin.

All volume measurements were made with a Bioquant IV morphometric system (R and M Biometrics, Inc., Nashville). The area of each ovary and corpora lutea was determined by circumscribing these structures in every tenth section. Ovarian volume was calculated with the following equation:

Ovarian volume (cubic millimeters) = Sum of section areas (in square millimeters) × 0.005 mm × 10

Volume measurements of individual corpora lutea and total corpora luteal volume per ovary were calculated with similar formulas. Statistical analysis was performed with one-way analysis of variance to compare the effect of varying doses of benzo(a)pyrene versus control at each week tested. When analysis of variance was used, the level of significance was noted by p, with values <0.05 considered significant. The unpaired Student t test was used to determine the significance of the effect of each individual dose. Because multiple comparisons were used, the Bonferroni correction was used to reduce the risk of type I error. Level of significance was noted by p with values <0.01 considered significant.

Results

Total ovarian volume. Over the 4-week period of the experiment, total control ovarian volume ranged from 0.8 to 0.9 mm^3 (Table I). One week after exposure, when varying doses were compared with control, benzo(a)pyrene significantly reduced ovarian volume ($p < 10^{-6}$). Compared with control ovarian volume, the 16% reduction in ovarian volume after 1 mg/kg benzo(a)pyrene was not significant. The remaining doses of 5 to 500 mg/kg produced highly significant ($p < 10^{-5}$), dose-dependent reductions in ovarian volume ranging from 67% to 35% of control. Two weeks after exposure, mean ovarian volume in mice treated with 5 or 10 mg/kg benzo(a)pyrene had returned to control levels. When varying doses were compared with control by analysis of variance, the benzo(a)pyrene-induced reduction in ovarian volume ($p < 10^{-6}$) remained significant among mice treated with benzo(a)pyrene at doses of 50, 100, and 500 mg/kg ($p < 0.01$, 10^{-6}, and 10^{-6}, respectively). The effect of

Table II. Total corpora lutea volume (in cubic millimeters, mean ± SD)

Dose (mg/kg)	*Week 1**	*Week 2†*	*Week 3*	*Week 4†*
Control	0.19 ± 0.04	0.12 ± 0.07	0.06 ± 0.05	0.11 ± 0.07
1	0.13 ± 0.11	0.12 ± 0.09	0.11 ± 0.09	0.11 ± 0.08
5	0.00 ± 0.00‡	0.13 ± 0.11	0.13 ± 0.10	0.18 ± 0.05§
10	0.01 ± 0.02‡	0.07 ± 0.12	0.09 ± 0.10	0.14 ± 0.05
50	0.00 ± 0.00‡	0.03 ± 0.06†	0.09 ± 0.11	0.12 ± 0.12
100	0.004 ± 0.01‡	0.02 ± 0.04‡	0.03 ± 0.07	0.01 ± 0.01†
500	0.00 ± 0.00‡	0.00 ± 0.00‡	0.00 ± 0.00†	0.00 ± 0.00†

*$p < 10^{-5}$.
†$p < 0.01$.
‡$p < 0.001$.
§$p < 0.05$.

the 1 mg/kg dose appeared significant ($p < 0.05$), but because p was not <0.01, this effect was not significant after the Bonferroni correction. Three and 4 weeks after exposure, when varying doses of benzo(a)pyrene were compared with control by analysis of variance, the benzo(a)pyrene-induced reduction in ovarian volume ($p < 10^{-5}$ and 10^{-6}, respectively) was significantly different from control only in the animals treated with the two highest doses, 100 mg/kg ($p < 0.05$ and 0.0001, respectively) and 500 mg/kg ($p < 10^{-4}$ and 10^{-6}, respectively). The apparent significance of the 100 mg/kg dose at week 3, however, was not maintained after the Bonferroni correction was applied.

Total corpora lutea volume. There appeared to be greater variation in total corpora lutea volume over the 4 weeks of this experiment than in total ovarian volume (Table II). Control corpora lutea volume ranged from 0.19 to 0.06 mm^3 over the 4 weeks. The lowest dose tested, 1 mg/kg, had no effect on corpora lutea volume, whereas the highest dose tested, 500 mg/kg, completely prevented corpora lutea formation. One week after exposure benzo(a)pyrene at doses >1 mg/kg effectively disrupted corpora lutea formation. Total corpora lutea volumes ranged from 0 to 0.01 mm^3, a highly significant reduction when compared with control by analysis of variance ($p < 10^{-6}$). A significant reduction in total volume persisted at 2 and 4 weeks after exposure, when all doses were compared with control by analysis of variance ($p < 0.002$ and 10^{-4}, respectively). Animals killed 2 weeks after benzo(a)pyrene exposure displayed a dose-dependent reduction in total volume at doses >10 mg/kg. The reductions were significant when compared with control for the 50, 100, and 500 mg/kg doses ($p < 0.01$, 0.001, and 0.001, respectively). Four weeks after treatment only the two highest doses, 100 and 500 mg/kg, produced a significant reduction in total volume ($p < 0.002$ and 0.01, respectively). Because of the relatively small corpora lutea volumes in the week 3 control animals, only the highest dose resulted in a significantly smaller volume than the control animals ($p < 0.01$).

Individual corpora lutea volume. Among control animals individual corpora lutea volumes varied from 0.023 to 0.039 mm^3 over the 4 weeks of this experiment (Table III). Individual corpora lutea volume, where corpora lutea were present an an ovary, appeared to be maintained 1 week after benzo(a)pyrene injection. At 2, 3, and 4 weeks after benzo(a)pyrene injection, there was a significant increase in mean individual corpora lutea volume in injected animals versus controls ($p < 10^{-4}$, 0.02, and 10^{-5}, respectively). This increase in individual volume was significant when corpora lutea formation recovered in the two lowest doses at week 2 ($p < 0.01$), all but the 5 mg/kg dose at week 3 ($p < 0.01$-0.001), and all but the 1 mg/kg dose at week 4 ($p < 0.01$ to 0.001).

Corpora lutea number. Over the course of the experiment total corpora lutea number in control animals varied from 2.5 to 5 corpora lutea per ovary (Table IV). When analysis of variance was used to compare the effect of multiple doses versus control at each week, there was a significant reduction in corpora lutea per ovary at 1, 2, 3, and 4 weeks afer benzo(a)pyrene treatment ($p < 10^{-6}$, 0.002, 0.02, and 10^{-4}, respectively). The highest dose, 500 mg/kg, completely prevented corpora lutea formation, whereas the lowest dose, 1 mg/kg, had no significant effect on corpora lutea numbers.

One week after benzo(a)pyrene treatment, doses ≥5 mg/kg disrupted corpora lutea formation, with only 0 to 0.5 corpora lutea per ovary ($p < 0.001$). By 2 weeks animals treated with benzo(a)pyrene at doses of 5 or 10 mg/kg began to recover their ability to form corpora lutea; however, the three highest doses, 50, 100, and 500 mg/kg, still exhibited a significant reduction in corpora lutea numbers ($p < 0.005$, 0.001, and 10^{-4}, respectively). Three weeks after treatment, recovery in corpora lutea number had occurred in all animals but

Table III. Individual corpora lutea volume (in cubic millimeters, mean ± SD)

Dose (mg/kg)	Week 1	Week 2*	Week 3†	Week 4*
Control	0.039 ± 0.014	0.030 ± 0.011	0.023 ± 0.010	0.027 ± 0.015
1	0.042 ± 0.012	0.038 ± 0.010‡	0.038 ± 0.018‡	0.035 ± 0.016§
5	—	0.037 ± 0.010‡	0.035 ± 0.022§	0.042 ± 0.013‖
10	0.028 ± 0.009	0.038 ± 0.016§	0.037 ± 0.012‖	0.035 ± 0.009‡
50	—	0.028 ± 0.007	0.040 ± 0.017‖	0.043 ± 0.015‖
100	0.039 ± 0.001	0.022 ± 0.005	0.039 ± 0.010‡	—
500	—	—	—	—

*$p < 10^{-4}$.
†$p < 0.02$.
‡$p < 0.01$.
§$p < 0.05$.
‖$p < 0.001$.

those injected with the two highest doses. A reduction in corpora lutea numbers persisted 3 and 4 weeks after treatment with 100mg/kg ($p < 0.05$ and 0.0005, respectively) and 500 mg/kg ($p < 0.002$ and 0.001, respectively). It should be noted that the effect of the 100 mg/kg dose was not significant at 3 weeks after the Bonferroni correction was applied.

Comment

Data among humans demonstrate that smoking impairs fertility. For example, studies by Baird and Wilcox[5] demonstrate that among women who are able to become pregnant within 2 years after stopping contraception the time to conception was considerably longer among smokers than nonsmokers. This effect appeared to be independent of socioeconomic status, history of reproductive disease, frequency of intercourse, and other factors previously identified as being associated with impaired fertility. A study of a different design by Vessey et al.[6] explored time to delivery among a group of women after cessation of contraception. This study demonstrated that the number of months until delivery of a child increased as a function of the number of cigarettes consumed.

In addition, an investigation conducted by MacMahon et al.[7] demonstrated that urinary estrogens during the luteal phase of the cycle were lower among smokers than nonsmokers. These data, collected in humans, suggest that if smoking impairs fertility, an impact on corpora lutea function may be responsible in part for this effect. Longcope and Johnston[8] reported no difference in serum levels of estrone and estradiol in premenopausal smokers versus controls. They suggested that the data of MacMahon et al.[7] indicate altered metabolism of estrogens rather than decreased production. Unfortunately, two factors may confound their data and make this conclusion premature. They failed to control for the menstrual cycle day when estrogens were measured. Given the large fluctuations in estrogens throughout the cycle, changes in estrogen production could easily be missed. In addition, the reduction in estrogen excretion reported by MacMahon et al. occurred only in the luteal phase. Follicular phase estrogen excretion was normal. This makes the failure of Longcope and Johnston to control for cycle day even more critical. It is also unlikely that estrogen metabolism would be radically different in the luteal versus follicular phase. Furthermore, the average daily consumption of cigarettes by the smoking group, although >10 per day, is not reported in detail. If cigarette smoking alters ovarian estrogen production in a dose-dependent fashion, this effect may be seen only in a group of heavy smokers. Given the limitations of this study, the effect of smoking on corpora lutea estrogen production is unclear.

In spite of the complex composition of cigarette smoke, however, no studies in humans to date have suggested the component (or components) contained in cigarette smoke that may be responsible for the adverse effects of smoking on reproductive performance. To further explore these issues we have conducted studies that have evaluated the impact of one smoke component on ovarian function in experimental animals. The focus of our experimental investigations has been the polycyclic aromatic hydrocarbons, a family of compounds contained in both direct and sidestream smoke. A recent report estimates the amount of benzo(a)pyrene in the mainstream smoke from a nonfilter cigarette to range from 20 to 40 ng. Up to 460 ng/hr can be inhaled in an environment containing cigarette smoke.[2] Previous investigations have demonstrated that exposure to polycyclic aromatic hydrocarbons destroys oocytes in murine ovaries.[9-12] This occurs in a time- and dose-dependent fashion and appears also to be a function of the strain and species of experimental animal used for the studies. In addition, previous studies have demonstrated that benzo(a)pyrene decreases fertility in rodents by decreasing ovulatory

Table IV. Corpora lutea number (No. per ovary, mean ± SD)

Dose (mg/kg)	*Week 1**	*Week 2†*	*Week 3†*	*Week 4**
Control	5.0 ± 1.3	4.0 ± 1.8	2.5 ± 1.5	3.9 ± 1.6
1	3.2 ± 2.6	3.0 ± 2.1	2.9 ± 1.6	3.2 ± 2.0
5	0.0 ± 0.0‡	3.5 ± 2.6	3.4 ± 1.8	4.4 ± 1.7
10	0.5 ± 0.8‡	1.8 ± 3.2	2.2 ± 2.2	3.9 ± 1.5
50	0.0 ± 0.0‡	1.0 ± 2.2§	2.3 ± 2.5	3.3 ± 2.7
100	0.1 ± 0.3‡	0.8 ± 1.8‡	0.8 ± 1.5‖	0.4 ± 0.7‡
500	0.0 ± 0.0‡	0.0 ± 0.0‡	0.0 ± 0.0§	0.0 ± 0.0‡

*$p < 10^{-4}$.
†$p < 0.02$.
‡$p < 0.001$.
§$p < 0.01$.
‖$p < 0.05$.

number and corpora lutea number.[4] The data presented in this study further define the impact of benzo(a)pyrene on ovulation and corpora lutea formation in mice.

Total ovarian volume, a composite measure reflecting the volume of growing follicles, antral follicles, corpora lutea, and stromal tissue, was reduced in both a time- and dose-dependent fashion in these studies. Corpora luteal volume averaged 14% of total ovarian volume in this study, consistent with the 17% previously reported from our laboratory.[13] To further define the effect of benzo(a)pyrene on corpora lutea, the dominant structure in the ovary after ovulation, we evaluated total corpora lutea volume, corpora lutea number, and individual corpora lutea volume. Over the 4-week course of this experiment there was a substantial reduction in number, which was both dose and time dependent. Interestingly, at 1 week after treatment, reductions in volume were seen at low doses of benzo(a)pyrene (5 mg/kg). Animals treated with doses of ≥100 mg/kg appeared to have a reduction in number throughout the course of this experiment. However, those animals treated wtih doses <100 mg/kg appeared to recover their ability to produce corpora lutea by the second to fourth week of this experiment.

It is of interest to note that if corpora lutea were present, individual corpora lutea volume did not decrease after benzo(a)pyrene administration. In fact, with recovery of the ability to form corpora lutea, individual corpora lutea after benzo(a)pyrene were significantly larger than control corpora lutea. This effect was seen even with the lowest dose studied (1 mg/kg). This compensatory hypertrophy of individual corpora lutea after recovery from benzo(a)pyrene is unable to compensate for the loss in number, however, and total volume is not maintained. Thus the fall in total corpora luteal volume in this experiment in a both dose- and time-dependent fashion is due to the reduction of corpora lutea number. Given the reduction in luteal urinary estrogens observed by MacMahon et al.[7] in female smokers, it is interesting to speculate that the reduction in total corpora lutea volume in these mice may result in reduced estrogen production. The lowered estrogen levels may result in reduced feedback at the pituitary and result in the individual corpora luteal hypertrophy. This concomitant increase in individual corpora lutea volume precludes proposing any hypothesis about corpora lutea estrogen production in a species, such as humans, that produces only one corpus luteum per cycle.

These data demonstrate more clearly the adverse impact of polycyclic aromatic hydrocarbons on ovarian function and corpora lutea formation. Even at doses of benzo(a)pyrene as low at 5 mg/kg reductions in total corpora lutea volume and number were observed in animals killed 1 week after exposure. There was a nonsignificant trend toward reduction of these parameters at the lowest dose used (1 mg/kg). Given that the reproductive system is dynamic and that benzo(a)pyrene is cleared from the animal over a period of several days, it is not surprising that there was resumption in corpora lutea formation over the 4-week duration of the experiment. The sensitivity of the ovary, however, to these low doses is of considerable interest because these doses, 1 to 5 mg/kg, appear to be substantially lower than the doses of polycyclic aromatic hydrocarbons responsible for induction of microsomal monooxygenases or other forms of toxicity, including carcinogenicity, that are typically ascribed to the polycyclic aromatic hydrocarbons.[14]

Two mechanisms may be responsible for the impairment in corpora lutea formation observed in this study. The antral follicle may rupture, but the granulosa cells may not luteinize because their function is impaired by benzo(a)pyrene, thus inhibiting corpora lutea formation. The ability of the benzo(a)pyrene-exposed ovaries to form a reduced number of hypertrophic corpora lutea before the recovery of corpora

lutea number and total volume suggests this cannot be the only mechanism. A more likely explanation is that corpora lutea formation is impaired because benzo(a)pyrene has destroyed follicles that would eventually mature, ovulate, and become luteinized to form corpora lutea. This hypothesis is supported by previous experiments that demonstrate that benzo(a)pyrene reduces follicle numbers in mice.[3]

The time- and dose-dependent loss in total corpora lutea volume and numbers (Tables II and IV) suggests which follicle types are destroyed by a given dose of benzo(a)pyrene. The recovery of corpora lutea formation by 2 weeks in the 5 mg/kg group suggests a direct effect on antral follicles. Corpora lutea formation was disrupted for 2 to 3 weeks in the 10 to 50 mg/kg groups. This suggests a direct effect on growing follicles, because primordial follicles require 21 days to reach the antral stage. All follicle types from primordial to antral are affected by the 100 and 500 mg/kg dose because the effect is immediate and persists past the length of time required for surviving primordial follicles to ovulate.

It should be pointed out that there are two potentially confounding factors in the design of this study. Intraperitoneal injections of the toxicant may allow direct uptake of large doses into the ovary and, hence, greatly increase toxicity. This effect is unlikely, however. Recent investigations in our laboratory demonstrated that direct intraovarian injection of benzo(a)pyrene caused no effect on corpora lutea formation in this strain of rodents. In contrast, intraovarian injection of several metabolites of benzo(a)pyrene resulted in inhibition of corpora lutea formation (unpublished data). In addition, previous experiments have demonstrated that it is possible to block the toxicity of benzo(a)pyrene to resting follicles with α-naphthoflavone, a compound that blocks the metabolism of benzo(a)pyrene.[15] This suggests that direct benzo(a)pyrene toxicity or intraovarian metabolism of benzo(a)pyrene is not an important aspect in its systemic toxicity on corpora lutea formation.

In addition, we did not control for cycle day or confirm the animals were cycling before injection. Previous studies have demonstrated that benzo(a)pyrene persists in these rodents for at least 7 to 10 days.[16-19] Because the compound persists for such a long time, at least two ovarian cycles in these rodents, we have assumed that cycle day of treatment is of little importance. The consistency and high degree of significance achieved in this study support this assumption because failing to control for cycle day should bias the results away from significance. The fact that fresh corpora lutea were identified in these animals at all time points, although frequently in reduced numbers, confirms that these animals indeed were ovulating.

Although reversibility of the benzo(a)pyrene-induced reduction of corpora lutea volume and number at doses <100 mg/kg suggests that there is resilience in the reproductive system, complete recovery is unlikely. The data in this investigation are consistent with the considerable destruction of resting and growing follicles by these doses of polycyclic aromatic hydrocarbons previously observed in studies by our group and others. The correlation between follicle numbers and the age at onset of reproductive failure[20] suggests that the total reproductive life span would be reduced in these animals.

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The mechanisms of preterm labor: Common genital tract pathogens do not metabolize arachidonic acid to prostaglandins or to other eicosanoids

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OBJECTIVE: Our aim was to determine the ability of pathogens commonly associated with genital tract infection and preterm labor to incorporate arachidonic acid and to metabolize it to prostaglandins or to other eicosanoids.
STUDY DESIGN: Four common genital tract pathogens, *Escherichia coli, Streptococcus viridans, Bacteroides fragilis*, and a group B β-hemolytic streptococcus, were incubated with tritium-labeled arachidonic acid for 2 to 48 hours. Uptake of arachidonic acid was calculated from uptake of radioactivity into the organisms. Tritium-labeled arachidonic acid within the medium was separated from any metabolites by high-performance liquid chromatography to assess metabolism of arachidonic acid within the bacteria.
RESULTS: Although all organisms were able to take up arachidonic acid, analysis of its metabolism with high-performance liquid chromatography demonstrated that none of these organisms will synthesize cyclooxygenase, lipoxygenase, or epoxygenase products.
CONCLUSION: Bacterial infection cannot initiate preterm labor by intrinsic biosynthesis and release of prostaglandins or other eicosanoids by the bacteria themselves. (Am J Obstet Gynecol 1992;166:1541-5.)

Key words: Preterm labor, infection, bacteria, arachidonic acid, prostaglandins, eicosanoids

There is a clear association between genital tract infection and the onset of labor before term.[1] Labor at term is associated with an increase in arachidonic metabolism within the uterus, in particular, within the amnion and decidua.[2] In amnion there is an increase in both cyclooxygenase metabolism and lipoxygenase metabolism, but the increase in cyclooxygenase metabolism predominates, producing a large increase in the synthesis of prostaglandin E_2 (PGE_2).[3] A similar increase in amnion prostaglandin synthesis has been demonstrated in association with preterm labor in the presence of infection.[4] Various hypotheses have been proposed for the mechanism by which bacterial infection increases arachidonic acid metabolism within the uterus. It has been suggested that release of phospholipases or endotoxins by bacteria may stimulate prostaglandin synthesis in the fetal membranes, or that a similar effect might be mediated by cytokines produced by inflammatory cells.[3, 5, 6]

Most attention has focused on the role of the cycloox-

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Table I. Retention data for arachidonic acid metabolites on reverse-phase high-peformance liquid chromatography

Compound	*Retention time (min)*	*Peak width (min)*
6-Keto-prostaglandin $F_{1\alpha}$	8.0	7.0-9.0
Thomboxane B_2	11.0	10.5-12.0
Prostaglandin $F_{2\alpha}$	13.0	12.5-14.5
PGE_2	16.5	15.5-18.0
13,14-Dihydro-15-keto-prostaglandin $F_{2\alpha}$	22.0	Not determined
13,14-Dihydro-15-keto-prostaglandin E_2	27.0	28.0-28.5
Prostaglandin A_2	29.0	Not determined
Prostaglandin B_2	31.5	Not determined
Leukotriene B_4	34.0	34.0-35.0
5-Hydroxyeicosatetraenoic acid (two peaks)	35.5	34.5-36.5
	43.0	42.0-44.0
11-Hydroxyeicosatetraenoic acid	42.5	43.0-44.0
12-Hydroxyeicosatetraenoic acid (two peaks)	35.5	35.0-36.0
	42.0	41.5-43.0
15-Hydroxyeicosatetraenoic acid	42.0	41.5-43.0
14,15-Hydroxyeicosatetraenoic acid	47.0	Not determined
Arachidonic acid	58.0	57.5-58.5

ygenase products of arachidonic acid, the PGs, in the initiation of preterm labor. Arachidonic acid can, however, be metabolized by lipoxygenase and epoxygenase enzyme pathways.[3, 7] The roles of these compounds in pregnancy and labor are not, at present, well understood, although we have shown that 5-hydroxyeicosatetraenoic acid is oxytocic.[8] Little attention has been paid to the possibility that the bacteria themselves might metabolize arachidonic acid to oxytocic compounds. Lipid fractions of *Propionibacterium acnes* have been found to have prostaglandin-like activity in bioassay, although production of prostaglandins cannot be demonstrated.[9] Gulbis et al.[10] originally reported production of PGE_2 by *Escherichia coli*. This appeared to be dependent on the presence of exogenous arachidonic acid and was inhibited by indomethacin. The same group later reported similar experiments[11] in which production of PGE_2 appeared to be independent of the presence of bacteria and in which indomethacin had no consistent effect. They concluded that their earlier work had been unreliable and that *E. coli* does not appear to synthesize prostaglandins.

We have therefore performed a series of experiments to determine whether common genital tract pathogens are able to take up exogenous arachidonic acid and whether they are able to syntheize cyclooxygenase, lipoxygenase, or epoxygenase metabolites.

Methods

Bacterial preparations. Single, pure colonies of laboratory stock isolates of four common genital tract pathogens, *E. coli, Streptococcus viridans, Bacteroides fragilis*, and a group B β-hemolytic streptococcus, were picked and incubated overnight at 37° C in a suitable medium (Oxoid No. 2 broth or Difco Thiol broth). This resulted in 10^8 organisms per milliliter of medium. The organisms were sedimented by centrifugation at 3000 rpm for 10 minutes and the medium was poured off. Fresh culture medium containing 0.2 μCi/ml tritium-labeled arachidonic acid was added to the organisms, and they were gently resuspended. The suspensions were divided into five aliquots of 10 ml each and incubated at 37° C.

Aliquots of suspensions of each of the organisms were incubated for 2, 4, 8, 24, and 48 hours. The organisms were then removed by centrifugation and filtration, and the bacterial pellet was retained to allow incorporated and unreleased radioactivity to be measured. Four milliliters of culture medium from each aliquot was stored at −20° C until further analysis. The remaining 1 ml of culture medium was added to scintillation fluid (Unisolve-1, Koch-Light, Ltd.) in the ratio 1:4, and radioactivity was counted to allow calculation of tritium-labeled arachidonic acid uptake by the bacteria. Each experiment was performed five times. Further experiments were performed in which the tritium-labeled arachidonic acid was supplemented by unlabeled arachidonic acid (10^{-9} mol/L) to determine the effect of concentration on uptake.

Control experiments were performed with bacterial culture medium containing 0.2 μCi/ml of tritium-labeled arachidonic acid without organisms.

Analysis of arachidonic acid metabolism by high-performance liquid chromatography. Each 4 ml aliquot of medium was acidified to pH 3.15 with formic acid and passed through a prepared C18 SepPak (Waters Associates), and the tritium-labeled arachidonic acid metabolites were eluted into 5 ml of methyl formate. The solvent was evaporated with oxygen-free nitrogen, and the residue was redissolved in 1 ml of 30% (vol/vol) acetonitrile in 40 mmol/L triethylamine formate. The fatty acids were then separated according to

polarity with a high-pressure liquid chromatography system based on a reverse-phase fatty acid analysis column. The initial solvent mixture was 30% acetonitrile in 40 mmol/L triethylamine formate, increasing to 50% and 100% acetonitrile after 25 and 52 minutes, respectively. The solvent flow was 1 ml/min, and 500 μl fractions were collected for a total of 60 minutes. Each fraction was dissolved in 4 ml of scintillation fluid, and radioactivity was counted in an Intertechnique SL3000 liquid scintillation counter. The radioactivity present in each metabolite peak in the chromatograms was calculated by summation.

We have previously reported the elution times for a wide range of arachidonic acid metabolites with this system[3, 7, 12] (Table I). Experiments have been performed in which radiolabeled prostaglandin $F_{2\alpha}$ and PGE_2 were added exogenously to culture medium to determine their recovery from the incubation medium. Recovery of these two compounds was found to be 94% (SD 2.3%) and 79% (SD 1.6%), respectively.[12]

Results

Each of the organisms incorporated tritium-labeled arachidonic acid, and the rates of incorporation and the total uptake were similar for all of the organisms. At 2, 4, 8, 24, and 48 hours, 21%, 32%, 38%, 52%, and 61%, respectively, of tritium-labeled arachidonic acid had been incorporated into the bacterial cells. Addition of unlabeled arachidonic acid had no effect on the proportion of tritium-labeled arachidonic acid taken up into the cells.

No significant peaks of radioactivity were detected at retention times corresponding to cyclooxygenase products in either experimental or control incubates. Significant peaks were detected at retention times corresponding to dihydroxyeicosatetraenoic acid and monohydroxyeicosatetraenoic acid (34, 35, and 42 minutes) and arachidonic acid (57.5 minutes) (Fig. 1). There was an increase in radioactivity corresponsing to dihydroxyeicosatetraenoic acid and monohydroxyeicosatetraenoic acid in both experimental and control incubates with increasing incubation time; however, this increase was significantly greater ($p < 0.01$) in control incubates (Fig. 2). There was a significant decrease in radioactivity corresponding to arachidonic acid in experimental incubates when compared with controls ($p < 0.01$) (Fig. 2).

Comment

Preterm labor remains a significant problem in obstetric practice. It accounts for up to 10% of all births but 85% of perinatal deaths in otherwise normal babies.[13] Although in many cases the cause of preterm labor remains obscure, there is a growing body of evidence that genital tract infection may play a role in

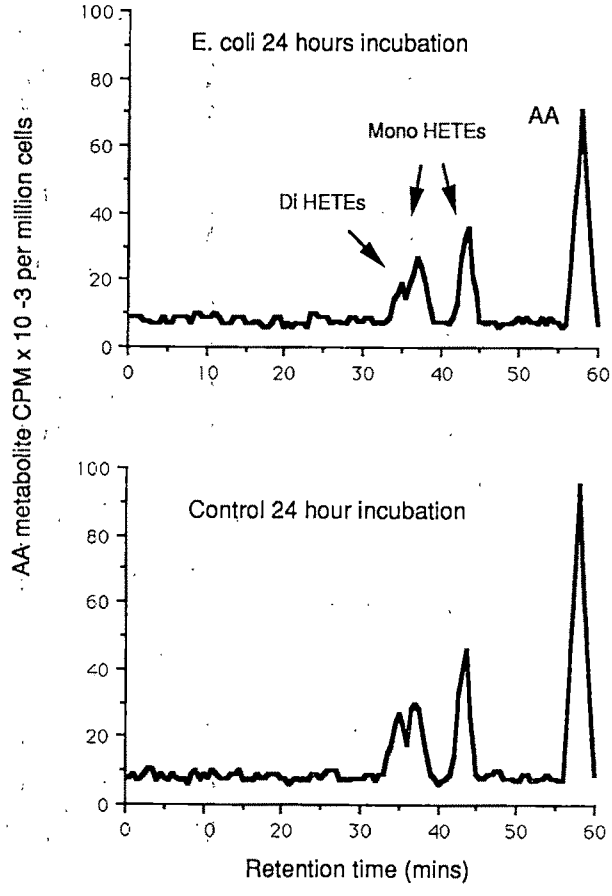


Fig. 1. Representitive high-performance liquid chromatography chromatograms showing tritium-labeled arachidonic acid metabolites found in culture after incubation of tritium-labeled arachidonic acid with *E. coli* for 24 hours *(top)* compared with control, sterile incubation. *AA*, Arachidonic acid; *HETE*, hydroxyeicosatetraenoic acid.

many cases. Several authors have reported an association between preterm rupture of membranes and maternal infection.[14] Others have demonstrated an association between preterm labor and infection with intact membranes, both with and without signs or symptoms of infection.[15] There is now a large body of evidence that an increase in prostaglandin synthesis within the uterus is an important early event in the onset of human parturition at term.[16] Labor is associated with increased synthesis of prostaglandins by the fetal membranes, in particular, the amnion. Curbello et al.[17] have found a high concentration of prostaglandin precursor arachidonic acid in amnion epithelial cells. In association with labor arachidonic acid metabolism in amnion increases with a large increase in the synthesis of PGE_2 and a smaller increase in metabolism through the lipoxygenase enzyme pathway to monohydroxyeicosatetraenoic acid and dihydroxyeicosatetraenoic acid and leukotriene B_4.[1, 2] Although the prostaglandins are known to be oxytocic and to mediate cervical ripening, little is

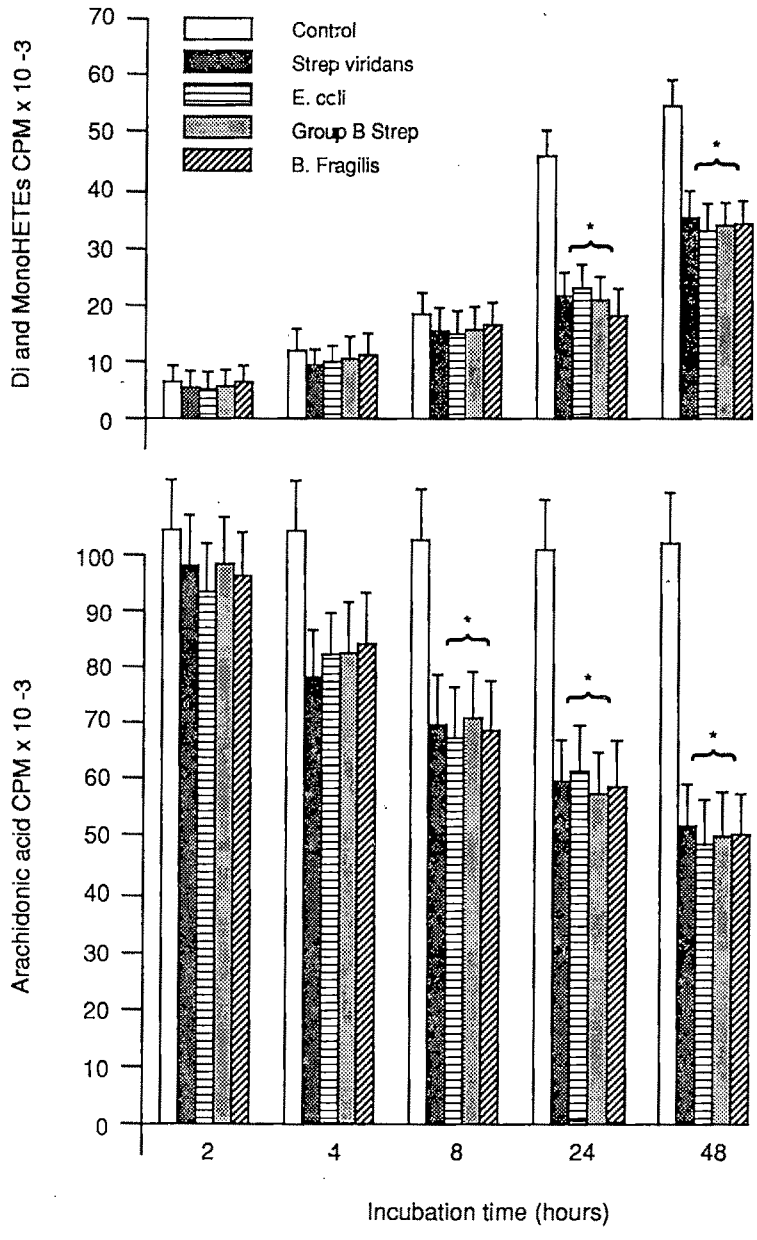


Fig. 2. Tritium-labeled arachidonic acid and dihydroxyeicosatetraenoic acid and monohydroxyeicosatetraenoic acid *(HETE)* found in culture medium after incubation of tritium-labeled arachidonic acid with various organisms between 2 and 48 hours. Mean values ± SD are shown for three different experiments in each case. Control incubations were sterile. *Asterisk*, $p < 0.05$, compared with control (analysis of variance).

known about the roles of the lipoxygenase metabolites of arachidonic acid. 5-Hydroxyeicosatetraenoic acid appears to be weakly oxytocic[7] and may play a role in the mediation of physiologic prelabor contractions.[18] Several substances found in amniotic fluid have been postulated to be fetally derived stimulators of arachidonic acid metabolism in the amnion; current candidates include platelet-activating factor[19] and interleukin-1β [20] Lopes-Bernal et al.[4] have reported a marked increase in PGE and leukotriene B_4 production from the amnion of preterm deliveries associated with chorioamnionitis, when compared with idiopathic preterm labor. Several theories have been proposed for the mechanism whereby infection increases prostaglandin synthesis by the amnion. Lamont et al.[21] and Bennett et al.[3] have shown that pathogenic bacteria release phospholipase, which causes changes in the metabolism of arachidonic acid in amnion similar to those changes seen with labor at term. Romero et al.[6, 20] suggest that it is the production of cytokines by inflammatory cells that stimulates prostaglandin synthesis by amnion.

In vitro studies of the effect of chorioamnionitis on

arachidonic acid synthesis have generally concentrated on the production of prostaglandins by the amnion. Little attention has been focused on the possibility that bacteria themselves may make prostaglandins or other eicosanoids. In this study we have investigated the ability of four common genital tract pathogens to metabolize exogenous arachidonic acid by cyclooxygenase, lipoxygenase, or epoxygenase enzyme pathways.

Although each of the organisms tested took up and incorporated arachidonic acid, there was no evidence that any of the four is able to metabolize that arachidonic acid by cyclooxygenase enzyme pathways to produce prostaglandins. Similarly there was no evidence of metabolism of arachidonic acid through epoxygenase enzyme pathways. Conversion of arachidonic acid to compounds that cochromatograph with dihydroxyeicosatetraenoic acid and monohydroxyeicosatetraenoic acid was seen; however, similar conversion was seen in bacteria-free control incubates. This implies that these compounds arise solely from autooxidation. The reduction in radioactivity corresponding to dihydroxyeicosatetraenoic acid and monohydroxyeicosatetraenoic acid in incubations containing bacteria when compared with controls was due to uptake of arachidonic acid into the bacteria, preventing its autooxidation. Radioactivity remaining within the organisms at the end of each experiment was measured to account for all of the radioactivity added at the start of each experiment. This excluded the possibility that the organisms are able to metabolize arachidonic acid to compounds that are not detectable with this system.

Our findings extend those of Gulbis et al.[10, 11] in demonstrating that common genital tract pathogens are unable to metabolize arachidonic acid to other eicosanoids by any of the established enzyme pathways. Arachidonic acid taken into bacterial cells may remain as a membrane component or be used as a source of energy. We have concluded that bacterial infection cannot initiate preterm labor in intrinsic biosynthesis and release of prostaglandins or other eicosanoids. Further work is needed to elucidate the precise mechanisms by which bacteria initiate preterm labor. It is likely, however, that a better understanding of the mechanisms of preterm labor will be gained by an increase in our understanding of the mechanisms of the onset of labor at term.

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Renal autoregulation in midterm and late-pregnant rats

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OBJECTIVES: The normal kidney autoregulates (that is, maintains) renal blood flow over a wide range of arterial blood pressures. During normal pregnancy blood pressure falls and the kidney vasodilates. The purpose of this study was to investigate whether renal autoregulatory ability was influenced during pregnancy in the normal rat.
STUDY DESIGN: Experiments were conducted on three groups of anesthetized rats, studied in the virgin state, at midterm, and late in pregnancy (gestation lasts 22 days in the rat). Renal blood flow was measured by electromagnetic flow probe at normal renal perfusion pressures, during elevation in renal perfusion pressure with bilateral carotid occlusion, and at subnormal renal perfusion pressures after application of an aortic snare.
RESULTS: Blood pressure was similar in midterm and virgin rats but lower in late pregnancy in the control state. Control renal blood flow was similar between virgin and late-pregnant rats but significantly elevated at midterm. During elevation in blood pressure with bilateral carotid occlusion, autoregulation was maintained in all three groups. During graded aortic occlusion, renal autoregulatory ability was lost. The autoregulatory threshold was between 90 and 100 mm Hg for midterm pregnant and virgin rats and was slightly lower at about 88 mm Hg for late-pregnant rats.
CONCLUSIONS: Midterm pregnant rats are able to autoregulate renal blood flow as well as virgins are in spite of underlying gestational renal vasodilatation. The slight shift in renal autoregulatory threshold seen in late-pregnant rats may prevent the kidney from hypoperfusion during late-gestation hypotension. (Am J Obstet Gynecol 1992;166:1546-50.)

Key words: Renal blood flow, pregnancy, autoregulation, rat

In normal pregnancy in women early increases occur in glomerular filtration rate and renal blood flow that reach a maximum of approximately 50% above the nonpregnant value.[1] In addition, total peripheral resistance declines so that in spite of concomitant plasma volume expansion the mean arterial blood pressure decreases by midterm.[1] In the normotensive pregnant rat qualitatively similar changes occur, although the temporal sequence is slightly different because the maximum decrease in renal vascular resistance occurs by midterm, whereas the decrease in arterial blood pressure occurs close to term.[2]

In the nongravid state renal function is preserved in the face of a decrease in arterial blood pressure by autoregulation of renal blood flow (and glomerular filtration rate), an intrinsic renal regulatory mechanism that maintains a constant blood flow (and filtration rate) over a wide range of arterial blood pressure.[3] Falls in arterial blood pressure below the autoregulatory range will, however, lead to renal hypoperfusion and a decline in glomerular filtration rate. Furthermore, reduced renal autoregulatory ability is seen even above the normal threshold for autoregulation, when the ambient tone in the renal vasculature is reduced with some but not all vasodilators.[3, 4] Because normal pregnancy is associated with early reductions in renal vascular tone and later reductions in arterial blood pressure, the current study was performed to investigate whether renal autoregulatory ability is altered in the pregnant rat. Studies were performed in anesthetized, volume-replete rats in the virgin state, at midterm (day 12), when the renal vasculature shows the maximal vasodilation but arterial blood pressure is normal, and in late pregnancy (day 19 to 20), when the renal vascular resistance is close to normal but gestational hypotension is evident.

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Methods

All experiments were performed with female Munich Wistar rats (aged 4 to 6 months) obtained from Simonsen, Gilroy, California. Rats were maintained on a 12-hour-light and 12-hour-dark cycle and had access to food (standard rat chow, containing ~24% protein and ~0.4% sodium) and tap water at will. Three groups were studied: midterm rats at day 12 of gestation, late-pregnant rats at days 19 to 20 of pregnancy (gestation lasts 22 days in the rat), and age-matched virgin females. Rats destined to become pregnant were placed together with a fertile male, and day 1 of pregnancy

was determined by the presence of sperm in the vaginal smears.

On the day of study rats were anesthetized by intraperitoneal injection of the long-acting thiobarbiturate Inactin (120 mg/kg body weight) (Byk Gulden Konstanz, Germany) and placed on a temperature-regulated surgery table; rectal temperature was maintained at 36° to 38° C. The left femoral artery was catheterized for periodic blood sampling and for monitoring arterial blood pressure (Gould P23 pressure transducer [Gould Inc., Oxnard, Calif.] connected to a Grass model 5D polygraph [Grass Instruments, Quincy, Mass.]) throughout the experiment. A femoral vein was also cannulated for infusion of isoncotic, artificial serum (2.5 gm/dl bovine serum albumin, 2.5 gm/dl bovine immunoglobulin in lactated Ringer's solution) at 12.5 ml/kg body weight per hour for 45 minutes during the preparatory surgery and thereafter at 1.5 ml/kg body weight per hour throughout the experiment. This protocol was effective in maintaining arterial hematocrit and plasma volume at preanesthesia levels during the preparatory surgery and throughout the experiment, thus preserving a euvolemic preparation.[5] Loose ligatures were placed around both carotid arteries, a tracheostomy was performed, and one jugular vein was catheterized for infusion of paraaminohippuric acid (0.5 gm/dl in 0.9% sodium chloride solution, 1 ml/hr). Midline abdominal and left subcostal incisions were made and a loose ligature was placed around the aorta above the level of both renal arteries. An electromagnetic flow probe (2 mm inner diameter, Carolina Medical Electronics, King, N.C.) was placed on the left renal artery, and renal blood flow was measured and recorded continuously (Grass model 5D polygraph). The left ureter was also cannulated for collection of urine during the control period only.

After a 60-minute equilibration period for paraaminohippuric acid infusion, a control period was performed during which an exactly timed (20-minute) urine sample was collected and a midpoint blood sample was drawn for measurement of renal blood flow (by paraaminohippuric acid clearance) to calibrate the flow probe. Renal blood flow was measured by electromagnetic flow probe only during the remainder of the experiment. After control, bilateral carotid occlusion increased arterial blood pressure, and renal blood flow and arterial blood pressure were measured during a 10- to 15-minute period. Although activation of efferent renal sympathetic nerve activity cannot be excluded, during carotid occlusion this most likely did not occur because the left kidney would be functionally denervated during dissection of the left renal artery and placement of the flow probe. Next, the ligatures around the carotid arteries were loosened and the ligature surrounding the aorta was tightened in a stepwise fashion to produce graded decreases in arterial blood pressure <90 mm Hg, in 10 mm Hg steps, down to 60 mm Hg. Each decrease in arterial blood pressure was followed by a 5-minute equilibration period and a 15- to 20-minute period during which renal blood flow and arterial blood pressure were measured.

At the termination of each experiment, zero blood flow was determined by occluding the renal artery on the side proximal to the kidney. Paraaminohippuric acid levels in urine and plasma samples during the control period were determined colorimetrically,[6] and clearance of paraaminohippuric acid was used to calculate renal plasma flow (and thus renal blood flow), assuming renal paraaminohippuric acid extraction to be 85%. The calculated renal blood flow (from paraaminohippuric acid clearance) obtained in the control period was then used to calibrate the flow probe. Before the study the flow probe was tested for linearity and stability with a dissected, suspended portion of the aorta and found to be linear in the range of 1 to 10 ml/min.

Autoregulatory curves of renal blood flow versus arterial blood pressure in control, with carotid occlusion and during graded aortic occlusions, were plotted for each animal. Values of renal blood flow at arterial blood pressures of exactly 80, 70, and 60 mm Hg were obtained by interpolation from individual animal autoregulatory curves. Statistical significance was determined by analysis of variance, and data are expressed as mean ± SE. In these experiments the West Virginia University guidelines for care and use of animals were followed.

Results

Body weight increases significantly during pregnancy, whereas kidney weights were the same in all three groups (Table I). Hematocrit fell progressively during pregnancy compared with the virgin values, reflecting the progressive gestational plasma volume expansion.[2] The uteri of all pregnant rats were inspected during the experiment and appeared normal for the stage of pregnancy.

During the control period there was no difference in arterial blood pressure between midterm and virgin rats, although a gestational renal vasodilation was evident with a renal blood flow approximately 20% greater in midterm rats (Fig. 1, $p < 0.05$). By late pregnancy significant falls had occurred in arterial blood pressure in spite of plasma volume expansion, which implies that total peripheral vascular resistance is markedly diminished close to term. In spite of the evidence for a peripheral vasodilation (i.e., fall in arterial blood pressure), renal vasodilation had abated by late in gestation and there was no difference in control renal blood flow between late-pregnant rats and virgin controls, (Fig. 1)

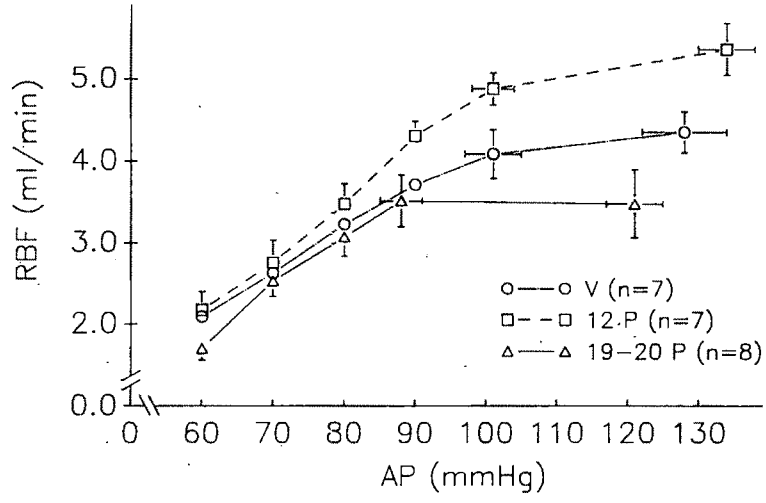


Fig. 1. Renal blood flow *(RBF)* at control blood pressure *(AP)*, at elevated arterial blood pressure during carotid occlusion and during reductions in arterial blood pressure by stepwise aortic occlusion in virgin (n = 7), midterm (12 days) (n = 7), and late-pregnant (19 to 20 days) (n = 8) rats. Mean ± SE for all renal blood flow measurements and for arterial blood pressure in control and during carotid occlusion. No standard errors are given for arterial blood pressure obtained during aortic occlusion because renal blood flow was determined at exactly 90 (virgins and midterm), 80, 70, and 60 mm Hg (all groups) by interpolation from individual animal autoregulatory curves.

Table I. Body weight, left kidney weight, and hematocrit in virgin (n = 7), midterm (n = 7), and late-pregnant (n = 8) Munich Wistar rats

Rats	*Body weight (gm)*	*Left kidney weight (gm)*	*Hematocrit (gm/dl)*
Virgins	202 ± 2	0.80 ± 0.01	47.5 ± 0.5
Midterm	236 ± 3*	0.85 ± 0.03	43.4 ± 0.6*
Late-pregnant	258 ± 4†‡	0.83 ± 0.03	40.6 ± 0.8†‡

All values are expressed as mean ± SE. Statistical significance was determined by analysis of variance.
*$p < 0.05$, midterm versus virgin rats.
†$p < 0.05$, late-pregnant versus virgin rats.
‡$p < 0.05$, midterm versus late-pregnant rats.

as previously reported for both the awake and anesthetized preparations.[7, 8] Renal blood flow was significantly lower in late-pregnant rats versus those in midpregnancy ($p < 0.01$).

Bilateral carotid occlusion produced ~30% increases in arterial blood pressure in all three groups (Fig. 1), and there was little effect on renal blood flow in virgin or pregnant rats. Thus differences in renal blood flow seen in control were maintained during carotid occlusion. During graded aortic occlusion reduction in arterial blood pressure to 90 mm Hg in virgins and midterm rats produced small decreases in renal blood flow (Fig. 1) of ~10% (Fig. 2). In late-pregnant rats control arterial blood pressure was ~90 mm Hg and control renal blood flow was similar to that in virgins and in midterm rats after arterial blood pressure reduction to 90 mm Hg. When arterial blood pressure was reduced <90 mm Hg, renal blood flow decreased in proportion to the decrease in arterial blood pressure in all three groups. There was no difference in renal blood flow among the groups at any arterial blood pressure.

Fig. 2 shows the percent change from control in renal blood flow (where control is 100%) during experimentally induced variations in arterial blood pressure. The inflection point of each curve represents the autoregulatory threshold, and, as shown, this threshold for virgin and midterm rats lies between 90 and 100 mm Hg. However, the autoregulatory threshold for late-pregnant rats is reset to a lower value of 88 mm Hg, which is the value of arterial blood pressure in control for these rats. In all groups autoregulation was in the arterial blood pressure range between control and bilateral carotid occlusion. At an arterial blood pressure between control and 90 mm Hg, there was impaired and variable autoregulation of renal blood flow in midterm rats, and renal autoregulation was absent in vir-

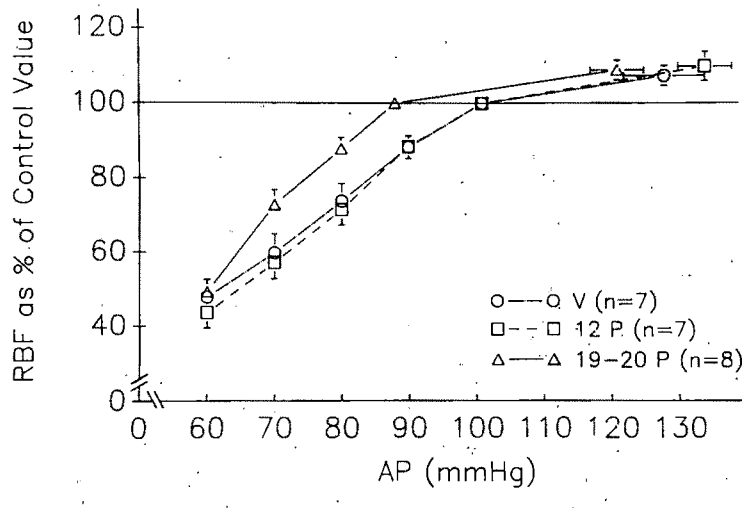


Fig. 2. Renal blood flow *(RBF)* during experimentally induced increases and decreases in arterial blood pressure *(AP)* expressed as percent of control renal blood flow. Data are given for virgins *(circles, dash-dot line)*, midterm *(squares, dashed line)*, and late-pregnant *(triangles, solid line)* rats.

gins. At values of arterial blood pressure <90 mm Hg, autoregulation was absent in all three groups (Fig. 2).

Comment

Several studies in nongravid animals have shown diminished autoregulation of renal blood flow during acute drug-induced systemic and renal vasodilatation.[4, 9] Selective renal vasodilation, by infusion of amino acids or hyperglycemia, also significantly impaired autoregulatory efficiency of renal blood flow.[10, 11] In pregnancy the kidney is chronically vasodilated, with glomerular filtration rate and renal blood flow reaching their gestational maximums early in pregnancy, and renal vasodilatation is sustained until near term, when glomerular filtration rate and renal blood flow return to prepregnant values.[1, 2] Our current study shows that, in contrast to the autoregulatory impairment seen with acute vasodilatation, with the chronic renal vasodilation of pregnancy there is apparently some resetting of the renal vasculature, so that an appropriate autoregulatory resistance change occurs in response to a change in pressure in spite of a chronic increase in vessel radius. In other words, the chronic gestational renal vasodilatation does not impair the autoregulatory ability of the kidney. Furthermore, in previous studies we have shown that the chronically vasodilated kidney of the midterm rat is also capable of responding with an appropriate acute renal vasodilatation in response to amino acid infusion.[12] One might expect that further vasodilatory responses would be attenuated in the already vasodilated renal vasculature of midterm pregnancy, but glycine infusion caused similar renal vasodilatation in both midterm and virgin rats.[12]

The results of our current study agree with a study by Woods et al.[13] in late-pregnant rabbits. These workers demonstrated similar autoregulation of renal blood flow and glomerular filtration rate between an arterial blood pressure of 70 and 100 mm Hg in virgins and in rabbits exhibiting a gestational renal vasodilatation in which renal blood flow was elevated by ~30%. However, one difference between their work and our study is that there was no gestational decrease in arterial blood pressure close to term in pregnant rabbits.[13] Studies by others in conscious, chronically catheterized rabbits disagree about whether rabbits demonstrate a late gestational hypotension,[13-15] and this variability probably depends on the time interval between catheterization and the experiment and the anxiety level of the rabbit during the experiment. By contrast, late pregnancy in rats is usually associated with gestational decreases in arterial blood pressure close to term regardless of whether the animals are studied under anesthesia or in the conscious, chronically catheterized preparation.[2, 7, 8] In our current study, by late pregnancy the gestational renal vasodilation has abated at a time when peripheral vasodilatation, plus opening of the uteroplacental shunt, had produced substantial falls in arterial blood pressure. Our findings indicate that the threshold for renal autoregulation changes close to term in pregnant rats and shifts to a slightly lower arterial blood pressure. If the autoregulatory threshold did not shift downward, the gestational decrease in arterial blood pressure would increase the risk of hypoperfusion of the maternal kidney. It is interesting that the arterial blood pressure of normal animals (whether pregnant or not) is close to the lower limit of autoregulation. Thus there is relatively little autoregulatory protection against renal hypoperfusion in the event of decreases in arterial blood pressure,

whereas autoregulatory mechanisms are most efficient at protecting the glomerulus from elevations in arterial blood pressure in the normal animal.

The current work does not address the mechanism(s) controlling renal autoregulation. It is currently believed that two major mechanisms control renal autoregulation, the myogenic and the tubuloglomerular mechanisms.[3] The myogenic theory states that changes in tone of the vascular smooth muscle of the afferent arteriole occur in response to changes in transmural pressure because of an inherent characteristic of the vessel. The tubuloglomerular feedback theory of autoregulation suggests that changing arterial blood pressure changes some signal that is sensed by the macula densa in the distal tubule, which in turn affects the resistance of the afferent arteriole of the same nephron. Woods et al.[13] believe that a tubuloglomerular feedback mechanism is responsible for renal autoregulation in pregnancy. Consistent with this hypothesis is our previous work in midterm rats in which we found that tubuloglomerular feedback activity is intact in spite of the gestational increase in plasma volume.[16]

In summary, we have found that autoregulation of renal blood flow is intact both in midterm rats when renal vasodilation is at its gestational maximum and arterial blood pressure is unchanged, and in late-pregnant rats when arterial blood pressure is decreased and renal vascular resistance is returning to the nonpregnant state. We have also shown that the autoregulatory threshold in late-pregnant rats is shifted toward lower arterial blood pressure, which may be a defense mechanism that helps prevent hypoperfusion of the maternal kidney when arterial blood pressure characteristically falls close to term.

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Effects of antenatal thyrotropin-releasing hormone, antenatal corticosteroids, and postnatal ventilation on surfactant mobilization in premature rabbits

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OBJECTIVE: The effects of antenatal hormones on postnatal surfactant mobilization were evaluated in preterm rabbits.

STUDY DESIGN: Pregnant rabbits were treated with vehicle, betamethasone, or thyrotropin-releasing hormone for 2 days before cesarean section at 29 days' gestation (term 31 days). Newborns were mechanically ventilated or allowed to spontaneously breathe, and groups were compared by analysis of variance.

RESULTS: Neither antenatal corticosteroids nor thyrotropin-releasing hormone increased radiolabeled precursor incorporation, alveolar wash or total lung saturated phosphatidylcholine pools, lung clearance of radiolabeled rabbit surfactant, or estimated net secretion of saturated phosphatidylcholine. However, saturated phosphatidylcholine pools in alveolar wash increased 2.7-fold during the first 24 hours in spontaneously breathing rabbits versus 2.1-fold in mechanically ventilated thyrotropin-releasing hormone–treated and control rabbits ($p < 0.05$). In addition, estimated net secretion of precursor-derived saturated phosphatidylcholine was 50% higher after 24 hours in spontaneously breathing rabbits.

CONCLUSION: Mechanical ventilation may have hindered the mobilization of surfactant saturated phosphatidylcholine pools to the alveolar space after birth in preterm rabbits, but maternal hormonal therapies did not appear to influence this adaptive process or change surfactant metabolism. (Am J Obstet Gynecol 1992;166:1551-9.)

Key words: Betamethasone, thyrotropin-releasing hormone, rabbits, lung development, pulmonary surfactant

Antenatal maternal administration of both corticosteroids and thyroid hormones may precociously induce lung maturation in preterm animals, as assessed by morphologic, physiologic, and biochemical criteria.[1, 2] Excellent reviews by Ballard[1] and Rooney[2] describe numerous studies in which both corticosteroids and thyroid hormones were associated with increased activities of the synthetic enzymes of surfactant saturated phosphatidylcholine in vitro, increased choline incorporation into saturated phosphatidylcholine in vitro, and increased lung lavage contents of saturated phosphatidylcholine in fetal rabbits before the onset of breathing. In contrast, when preterm rabbits at 27, 28, or 29 day's gestation were mechanically ventilated for 30 to 60 minutes before having their lungs lavaged, the saturated phosphatidylcholine pools recovered from the lavages were no longer increased after the antenatal administration of corticosteroids or thyrotropin-releasing hormone.[3-6] However, these apparently conflicting results would be compatible if the prenatal stimulation of synthesis or secretion after hormonal therapy was offset by an equivalent inhibition of secretion or increase in reuptake after birth and the onset of ventilation. The individual components of surfactant saturated phosphatidylcholine metabolism (secretion, reuptake, synthesis, and clearance) have not been previously studied postnatally in mechanically ventilated preterm animals after maternal corticosteroids or thyroid hormones are given antenatally.

For several reasons we chose to study preterm rabbits at 29 days' gestation whose mothers received hormonal therapy on the twenty-seventh and twenty-eighth days of gestation. First, the rates of surfactant secretion and recycling are very high in the lungs of preterm rabbits delivered at 29 days' gestation as demonstrated by the 30-fold increase that occurred in their alveolar phosphatidylcholine pools from delivery to 24 hours after the onset of spontaneous breathing.[7] Second, rabbits at this gestational age in our laboratory still had significant surfactant deficiency, with alveolar pool sizes of <10 μmol saturated phosphatidylcho-

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line/kg body weight after 30 minutes of ventilation,[6] and a need for supplemental oxygen for survival (unpublished data). Third, we were able to maintain rabbits at this gestational age on mechanical ventilation for up to 24 hours, enabling randomization of large litters to various treatments at multiple time points. Finally, when preterm rabbits at various gestational ages received corticosteroids by maternal administration during the 2 days before delivery, the maximal effect on protein leak and lung function, in the absence of exogenous surfactant replacement, was seen when the mother was treated at 27 and 28 days' gestation and the fetuses were delivered at 29 days.[6]

Methods

Maternal corticosteroid and TRH treatment. Pregnant New Zealand White rabbits were injected with either saline vehicle or 0.1 mg/kg betamethasone (Celestone Soluspan, Schering, Kenilworth, N.J.) given intramuscularly 48 and 24 hours before delivery, or 0.02 mg/kg TRH (Bachem, Torrance, Calif.), given intravenously 48,36, 24, and 12 hours before delivery on the twenty-ninth gestational day. Vehicle control, corticosteroid-treated, and TRH-treated litters were studied on the same day. Vehicle control and TRH-treated litters were either allowed to spontaneously breathe or were tracheostomized and mechanically ventilated. All corticosteroid-treated litters were mechanically ventilated due to the lower birth weights and increased lability of the rabbits from those litters. Similarly, exclusion criteria were selected so that all the animals analyzed were in comparable pH balance (see Data analysis). All guidelines for the care and use of the rabbits approved by our institution were followed.

Radioisotope and surfactant preparation. Palmitic acid (1-carbon 14, 56 Ci/mol) and phosphorus 32 (carrier-free, as orthophosphate) were purchased from ICN Pharmaceuticals (Irvine, Calif.). The palmitic acid was converted to the sodium salt and stabilized with 6% bovine serum albumin.[8] These isotopes were then combined in a 0.9% sodium chloride solution containing 15 μCi/ml [^{14}C]palmitic acid and 80 μCi/ml [^{32}P]orthophosphate and kept frozen until use.

Unlabeled natural rabbit surfactant was prepared from the pooled lung lavages of adult rabbits, and the large surface-active aggregates were isolated by centrifugation of the alveolar wash at 8000 *g* for 30 minutes at 4° C over 0.7 mol/L sucrose.[9] The large aggregates recovered at the interface were pelleted at 40,000 *g* and resuspended in 0.9% sodium chloride solution for later use. The rabbit surfactant was then radiolabeled by mixing it with a suspension of liposomes made with [^{3}H]choline-labeled dipalmitoylphosphatidylcholine.[10] The mixture was centrifuged at 27,000 *g* for 15 minutes, and the pellet was suspended in 0.45% sodium chloride solution to obtain a final concentration of 1.3 μCi/ml and 1 mg dipalmitoylphosphatidylcholine/ml. Liposomes made in this manner and associated with natural-surfactant large aggregates were shown previously to have the same lung clearance kinetics as natural surfactant in 3-day-old rabbits.[10]

Delivery and ventilation. On the twenty-ninth day of gestation the does were anesthetized with intravenous pentobarbital and given supplemental oxygen by face mask. Local anesthesia with 1% lidocaine was given in the abdominal wall, followed by exposure of the uterus and sequential delivery of the rabbit fetuses. Each newborn was then weighed, and those that were tracheostomized and mechanically ventilated were given a mixture of 10 mg/kg ketamine and 0.1 mg/kg acepromazine intraperitoneally. Those newborns randomized to the mechanically ventilated groups were tracheostomized with a tube made from an 18-gauge needle and given 150 μl of the preparation of tritium-labeled rabbit surfactant. This low dose of surfactant was administered after lightly compressing the chest until tracheal fluid completely filled the endotracheal tube and was immediately followed by the administration of several breaths of 100% oxygen with an anesthesia bag. By 3 minutes of age each rabbit was transferred to a temperature-controlled ventilator-plethysmograph with each set of 10 ventilator circuits driven by a time-cycled, pressure-limited Sechrist infant ventilator (Sechrist Industries, Inc., Anaheim, Calif.).[4] Each rabbit was then continuously ventilated with 50% oxygen at a rate of 30 breaths/min with an inspiratory time of 0.7 seconds and no positive end-expiratory pressure. Peak inspiratory pressure was regulated individually every 20 to 30 minutes with a series of water columns to maintain the tidal volume at 12 ml/kg body weight, as measured with a pneumotachometer.[4] This amount of ventilation was found to result in stable compliances and normal P_{CO_2} values when the rabbits were killed. Dynamic compliance measurements were calculated by dividing tidal volume/kg body weight by peak inspiratory pressure. After delivery of the spontaneously breathing control and TRH-treated litters, the newborn rabbits were thoroughly dried, stimulated, and placed in an incubator at 37° C with 50% oxygen for 30 minutes before the intratracheal and intravenous injections of radioisotopes. After 30 minutes, the rabbits were injected intratracheally with 150 μl of the preparation of tritium-labeled rabbit surfactant with a 27-gauge needle.[11]

Each newborn rabbit was injected through the external jugular vein with 0.2 ml of the [^{14}C]palmitate and [^{32}P]orthophosphate surfactant precursor solution 10 minutes after receiving the intratracheal surfactant. Each rabbit was fed 20 to 30 minutes after birth and every 8 hours thereafter with 1 to 1.5 ml of Esbilac

Table I. Description of groups of animals

	No.	Body weight* (gm)	Tidal volume* (ml/kg)	Lung clearance of tritiated rabbit surfactant	
				%/hr	r†
Control mechanical ventilation	37	38.6 ± 5.7	12.1 ± 1.1	0.8	0.88
Steroid mechanical ventilation	29	34.0 ± 3.7‡	11.9 ± 1.4	0.9	0.61
TRH mechanical ventilation	28	38.0 ± 4.4	12.5 ± 0.8	0.4	0.63
Control spontaneously breathing	45	38.4 ± 6.3		0.8	0.68
TRH spontaneously breathing	38	37.9 ± 6.9		1.0	0.96

*Group mean ± SD.

†Used to calculate clearance.

‡$p < 0.05$ versus control and TRH.

formula (Pet-Ag, Inc., Hampshire, Ill.) through a 3.5Fr orogastric infant feeding tube. This feeding schedule successfully prevented hypoglycemia as measured by Dextrostix on terminal blood samples. Oxacillin, 50 mg/kg, was given intramuscularly at 2 and 14 hours of life. Rabbits from each litter were killed with intrathecal injections of lidocaine at 1, 4, 8, 15, and 24 hours after receiving the intravenous doses of radiolabeled precursors, and terminal blood samples were drawn from the hearts for blood gas analysis.

Processing of lungs. The chest of each rabbit was opened and five aliquots of sufficient 0.9% sodium chloride solution to distend the lungs (2 to 3 ml) were flushed into and out of the airways three times for each aliquot and then pooled as the alveolar wash before storage at −20° C for further analysis. The postlavage lung tissue was then removed from the chest, weighed, and homogenized in 4 ml sodium chloride solution before storage at −20° C as the lung homogenate. Lipids from aliquots of the alveolar washes and lung homogenates and from duplicate samples of the intratracheal injection solutions were extracted with chloroform/methanol (2:1) and dried under nitrogen at 50° C.[12] The lipid extracts were then treated with osmium tetroxide and the saturated phosphatidylcholine was recovered from the extracts by column chromatography with neutral alumina according to Mason et al.[13] Each saturated phosphatidylcholine sample was then split and analyzed for phosphorus content according to Bartlett[14] and radioactivity by liquid scintillation counting with Aquasol II (New England Nuclear, Boston).

Data analysis. Rabbits were excluded from analysis for terminal pH values <7.00 or P_{CO_2} values >65 mm Hg. Results are reported as group means ± SEM unless otherwise indicated. The rates of clearance of the radiolabeled intratracheal surfactant preparations were calculated from the slopes of the linear regression curves of the recovery percentages. Differences between hormonal treatment groups were tested by analysis of variance followed by the Student–Newman-Keuls multiple comparison procedure. Differences between mechanically ventilated and spontaneously breathing rabbits for the TRH-treated and vehicle control litters were tested by two-way analysis of variance.

Results

Description of animals. Of the mechanically ventilated preterm rabbits, 77% of the control group, 72% of the TRH group, and 46% of the betamethasone group satisfied the pH and P_{CO_2} criteria and were included in the results. Metabolic acidosis and intravascular volume depletion by clinical assessment accounted for the vast majority of those excluded from analysis, regardless of the treatment group. The significantly higher exclusion rate in the newborns from the betamethasone group was probably a secondary effect of smaller intravascular volumes at birth after their intrauterine growth retardation. Of the spontaneously breathing rabbits, 96% of the control group and 86% of the TRH group met the inclusion criteria. The number of animals included in the results for each group and for body weights and mean tidal volumes during mechanical ventilation are shown in Table I. Maternal corticosteroid treatment resulted in a significant reduction in fetal body weight ($p < 0.05$), but peak pressures were adjusted accordingly so that mean tidal volumes were comparable in all the mechanically ventilated rabbits. The group means and SDs for pH and P_{CO_2} values obtained from the terminal blood gases from all the rabbits analyzed and for the mean peak inspiratory pressures and dynamic compliances of the mechanically ventilated rabbits analyzed are shown in Fig. 1. Neither antenatal maternal TRH nor betamethasone resulted in any significant differences in those animals that met the inclusion criteria. The dynamic compliances measured in the mechanically ventilated rabbits tended to improve with time. All rabbits initially had some degree of metabolic acidosis which seemed to improve over time and there was a somewhat higher

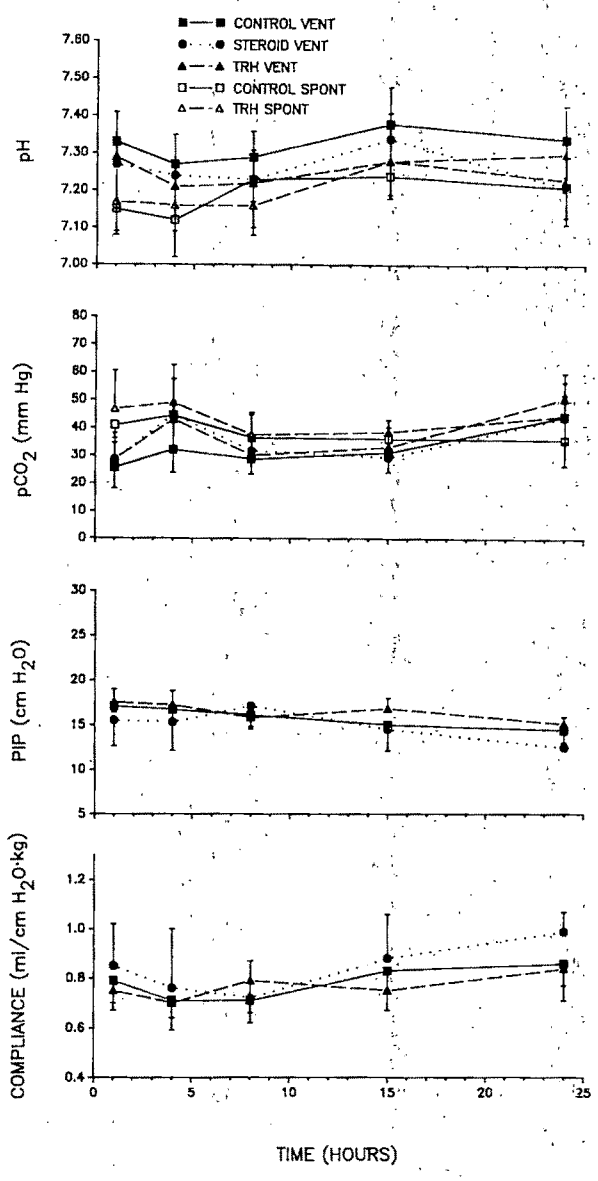


Fig. 1. pH, P_{CO_2}, peak inspiratory pressure *(PIP)*, and dynamic compliance values for those rabbits meeting inclusion criteria. Each point represents group mean ± SD for animals killed at that time. *Solid symbols*, Mechanically ventilated rabbits; *open symbols*, spontaneously breathing rabbits. Antenatal hormone treatments are represented by *different symbols* as indicated. Dynamic compliance and peak inspiratory pressure were only measured in mechanically ventilated rabbits.

degree of respiratory compensation after 1 hour in those rabbits that were mechanically ventilated.

Lung clearance of intratracheal saturated phosphatidylcholine liposomes associated with rabbit surfactant. Tritiated choline dipalmitoylphosphatidylcholine liposomes associated with natural rabbit surfactant were cleared from the total lungs (alveolar wash ± lung homogenate) of all groups at very slow rates of ≤1%/hour from 1 to 24 hours postnatally as calculated by linear regression of the total lung recovery curves (Table I). There were no significant differences resulting from either maternal hormone treatments or the use of mechanical ventilation.

Lung incorporation of saturated phosphatidylcholine precursors. ^{14}C-labeled palmitate was incorporated more rapidly and more efficiently into preterm rabbit lung saturated phosphatidylcholine (Fig. 2, *A*) than [^{32}P]orthophosphate, as expected (Fig. 2, *B*), because the former may enter the fatty-acid side chains of saturated phosphatidylcholine by remodeling or de novo synthesis whereas the latter must be incoprorated into the saturated phosphatidylcholine backbone by de novo synthesis. Because incorporation through the remodeling pathway is very rapid and probably more efficient than incorporation through de novo synthesis, the maximum incorporation for ^{14}C-labeled palmitate occurs by 1 hour, after which clearance of labeled saturated phosphatidylcholine equals or exceeds incorporation by de novo synthesis. However, the incorporation of the precursors was not significantly affected by maternal TRH, maternal betamethasone, or mechanical ventilation.

Estimated secretion of saturated phosphatidylcholine. Secretion of lung saturated phosphatidylcholine into the air spaces after birth was estimated by measuring the percentage of ^{14}C-precursor–labeled saturated phosphatidylcholine recovered from the alveolar wash relative to the recovery from the total lung (Fig. 3). As expected, the percentage of precursor-labeled saturated phosphatidylcholine recovered from the alveolar wash increased significantly over time in all groups. Neither maternal TRH nor corticosteroids caused any significant changes in the percentages of labeled saturated phosphatidylcholine recovered from the washes of the newborns at any of the time points studied. However, all groups of rabbits that were mechanically ventilated had significantly smaller percentages of precursor-labeled saturated phosphatidylcholine recoveries from their alveolar washes after 24 hours than did rabbits allowed to spontaneously breathe ($p < 0.05$).

Saturated phosphatidylcholine pool sizes. Saturated phosphatidylcholine pools recovered from the air spaces by alveolar wash and from the remaining lungs after homogenization increased significantly in all five groups from 1 to 24 hours ($p < 0.01$), but neither maternal TRH nor corticosteroid treatment resulted in significantly increased alveolar wash or lung homogenate saturated phosphatidylcholine pools compared with controls (Fig. 4, *A* and *B*). However, although neither lung homogenate (Fig. 3, *B*) nor total lung (alveolar wash + lung homogenates) saturated phosphatidylcholine pools (data not shown) were changed significantly by the mode of ventilation, the alveolar wash saturated phosphatidylcholine pools increased 2.1-fold postnatally from 1 to 24 hours in mechanically ventilated TRH and control groups compared with 2.7-fold in spontaneously breathing control and TRH groups

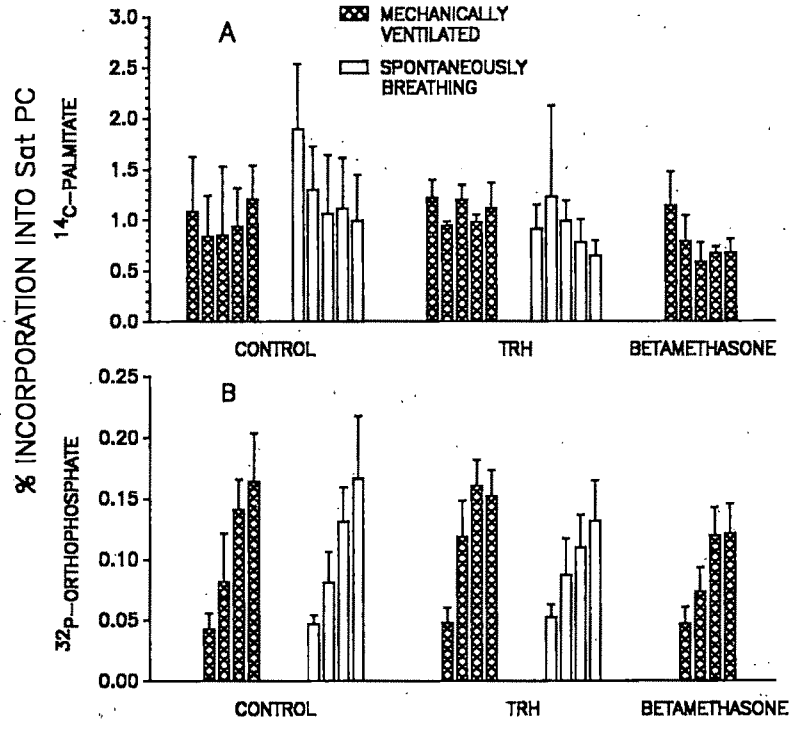


Fig. 2. Percentage of administered radiolabeled precursors incorporated into preterm rabbit lung saturated phosphatidylcholine *(Sat PC)* from 1 to 24 hours. All incorporation percentages refer to recoveries from total lungs (alveolar wash plus post lavage lung tissue). In **A** *bars* from left to right within each treatment group represent group means ± SE for rabbits killed at 1, 4, 8, 15, and 24 hours. Antenatal hormone treatments are indicated below *x* axis. *Cross-hatched bars,* Mechanically ventilated rabbits; *open bars,* spontaneously breathing rabbits. In **B** results are shown for rabbits killed at 4, 8, 15, and 24 hours because amount of ^{32}P incorporated by 1 hour was very low.

($p < 0.05$ by two-way analysis of variance), which was consistent with the lower estimated secretory rates and unchanged incorporation rates seen in those groups that were mechanically ventilated.

Lung uptake and recycling of surfactant saturated phosphatidylcholine. The fractional recovery from the alveolar wash of the tritiated saturated phosphatidylcholine that was given intratracheally after being associated with natural rabbit surfactant decreased initially consistent with reuptake but then actually had a tendency to increase during the later times, consistent with resecretion and increasing mobilization of alveolar pools (Fig. 5). This progression was not affected by maternal hormone treatments, but the spontaneously breathing controls and TRH-treated rabbits tended to have the largest increases in fractional recoveries from the alveolar washes at the later times.

Comment

Rooney et al.[15] found in preterm rabbits (27 days' gestation) that maternal TRH, at the same dose as in the current study (0.02 mg/kg for four doses), increased the alveolar pool sizes of phosphatidylcholine when lung lavages were performed before the onset of ventilation, whereas Ikegami et al.[5] found no significant change in alveolar pools compared with vehicle-injected controls when the lung lavages were done after 30 minutes of mechanical ventilation. Similarly, Fiascone et al.[3] recovered increased alveolar pools of surfactant phosphatidylcholine from unventilated preterm rabbit lungs after the administration of antenatal maternal corticosteroids when compared with the newborns of vehicle-injected controls but recovered much larger alveolar phosphatidylcholine pools with no differences between the offspring of corticosteroid-treated and controls after 1 hour of mechanical ventilation. However, an unchanged alveolar pool size may occur in spite of significant alterations in surfactant metabolism because synthesis, secretion, recycling, and clearance or degradation all contribute in a complicated manner to the resulting alveolar pools recovered.[16, 17] This is especially true near term during neonatal transition when both secretion into and reuptake from the alveolar space rapidly increase and then gradually reequilibrate to a new set point.[16] The alveolar pools of preterm rabbits at 29 days' gestation increased 30-fold from the time of delivery until 24 hours after the onset of spontaneous breathing,[7] and the alveolar pools of mechanically ventilated preterm lambs at 136 days' gestation increased fourfold from birth to 20 minutes of age and

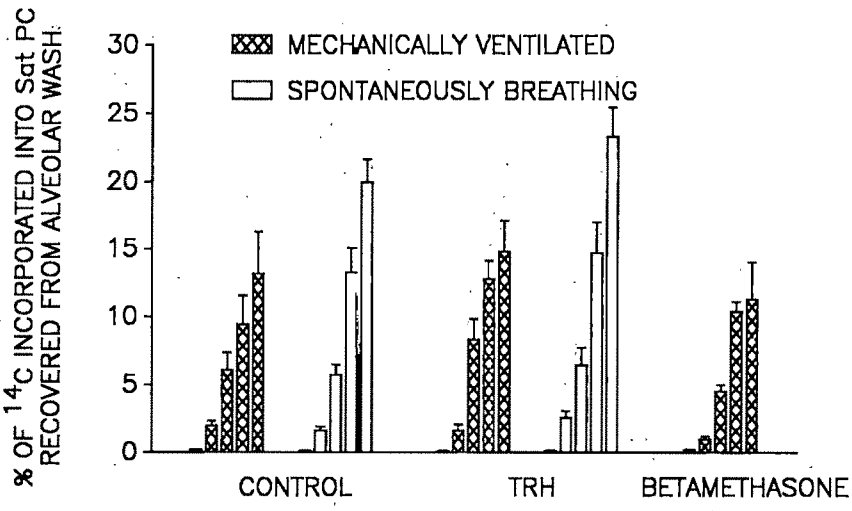


Fig. 3. Effects of various treatments on estimated net secretion of precursor-labeled saturated phosphatidylcholine *(Sat PC)* from 1 to 24 hours. Bars from left to right within each treatment group represent group means ± SE for rabbits killed at 1, 4, 8, 15, and 24 hours with almost no secretion seen in all groups at 1 hour. *Cross hatched bars,* Mechanically ventilated rabbits; *open bars,* spontaneously breathing rabbits. Antenatal maternal therapy is indicated below *x* axis under corresponding groups.

doubled again by 10 hours of age.[18] We studied preterm rabbits delivered at 29 days' gestation to measure the postnatal effects of hormonal agents and mechanical ventilation on surfactant saturated phosphatidylcholine metabolism during this period of rapid mobilization of surfactant saturated phosphatidylcholine pools into the air spaces.

Hormonal effects on lung maturation and surfactant metabolism are related to dose, species, the time in gestation during which they are administered, and whether they are given to the mother or the fetus.[19-25] Direct fetal administration of corticosteroids in lambs and rabbits appeared to cause more pronounced effects on surfactant phosphatidylcholine pools and smaller effects on overall fetal growth,[20,23-26] whereas maternally administered corticosteroids in rabbits and primates often did not cause any significant changes in phosphatidylcholine pool sizes in the fetuses but did inhibit growth in preterm rabbits.[4,5,19,23,27] Evaluating thyroid hormone effects on lung function and surfactant phospholipid metabolism is further complicated by interspecies differences in placental transport of thyroxine and triiodothyronine with particularly limited maternal-fetal transport across the human placenta. Thyroxine injected intraamniotically did appear to accelerate fetal lung maturation but required amniocentesis for administration.[28,29] TRH is clinically a potentially useful preparation because it readily crosses the human placenta and when given intravenously to mothers with term fetuses caused significant increases in cord blood levels of thyroid-stimulating hormone, triiodothyronine, and thyroxine 2 hours later.[30,31] The elevation of triiodothyronine and thyroxine in fetal blood was transient and was not seen 12 hours after maternal TRH administration in preterm rabbits[5] or humans.[30] Liggins et al.[32] found significant increases in alveolar pool sizes of saturated phosphatidylcholine at birth in preterm lambs given fetal infusions of TRH alone compared with controls and those given cortisol alone, which were not different from each other. Oulton et al.[24] found no significant increases in the alveolar phospholipid pools of 27-day preterm rabbits at birth after 0.04 mg/kg per dose of maternal TRH given intravenously in single or multiple doses (24 hours apart) from 24 to 26 days' gestation. However, the preterm rabbits all had increased lamellar body (intracellular) fractions of phospholipids compared with controls whose mothers did not receive TRH antenatally. These increased lamellar body pools would theoretically create a larger pool from which saturated phosphatidylcholine could be mobilized during neonatal transition, but increased mobilization after maternal TRH administration was not seen in 27-day preterm rabbits after 30 minutes of ventilation[5] in spite of larger alveolar pools seen at 27 days before the onset of ventilation.[15] This suggested to us that antenatal thyroid hormones might also have effects on postnatal secretion and recycling and that 29 days' gestation was a good time to study these phenomena because of the very high rate of mobilization previously seen at this point of development in spontaneously breathing rabbits.[7]

Maternal TRH administration might also stimulate lung maturation by increasing the release of prolactin or by direct central effects on respiratory timing because it has been shown to increase fetal breathing movements in lambs in utero[33] and to increase respiratory rate and minute ventilation in preterm rabbits spontaneously breathing at 29 days' gestation.[34] We

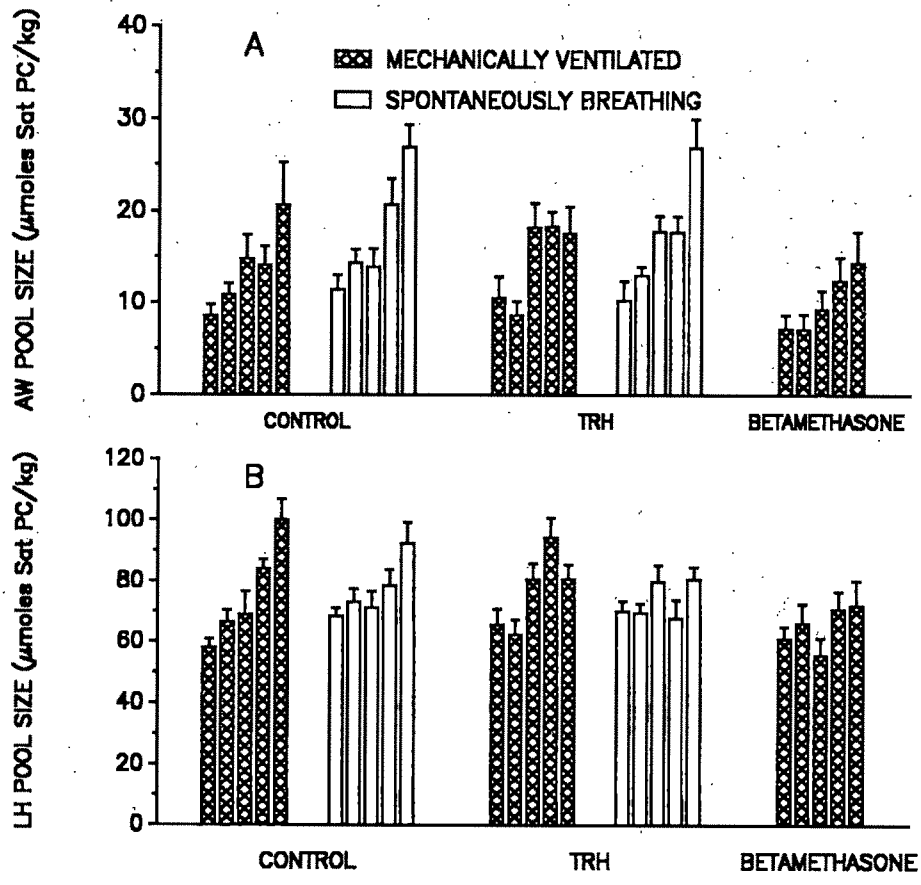


Fig. 4. Surfactant saturated phosphatidylcholine *(Sat PC)* pool sizes recovered from air spaces by alveolar wash *(AW)* in **A** and from remaining lung homogenates *(LH)* in **B** as determined by measuring total phosphorus content of saturated phosphatidylcholine fraction. Bars from left to right within each treatment group represent group means ± SE for rabbits killed at 1, 4, 8, 15, and 24 hours. Group means are expressed as micromoles saturated phosphatidylcholine per kilogram of body weight. Ventilatory style and antenatal hormonal therapy are as in previous figures. Proportion of total lung saturated phosphatidylcholine pool (alveolar wash plus lung homogenates) that was recovered from alveolar wash increased significantly over time in all groups ($p < 0.01$).

therefore studied the effects of matenal TRH on both mechanically ventilated and spontaneously breathing preterm rabbits and compared them with similarly ventilated controls. Because respiratory timing effects have not been reported after corticosteroid treatments and the newborns were smaller and generally more labile, we only studied mechanically ventilated rabbits for that treatment. However, in this study antenatal maternal TRH did not appear to affect surfactant saturated phosphatidylcholine metabolism in either spontaneously breathing or mechanically ventilated rabbits. Most of the previous studies had used much higher doses of TRH by direct fetal injection either intracerebrally or intravenously.[33, 34]

Total lung incorporation of radiolabeled palmitate and orthophosphate precursors into saturated phosphatidylcholine was not consistently affected by either hormonal treatments nor ventilatory style. Although it is possible that concurrent changes in precursor pool sizes of fatty acids and phosphate could have obscured any hormonal effects, it is unlikely that this occurred because the incorporation changed with time in a consistent and appropriate manner for two different precursors entering different pools. In addition, total lung saturated phosphatidylcholine pool sizes per kilogram body weight were also similar after TRH, corticosteroid, or vehicle administration. This strongly supports the existence of similar synthetic rates for saturated phosphatidylcholine because the contribution of lung clearance to the pool sizes was minor with <1% per hour cleared from the lungs in all the groups. The relatively small changes that occurred in parenchymal lung pools of saturated phosphatidylcholine after birth resulting from relatively little saturated phosphatidylcholine synthesis and very little clearance were consistent with the stable parenchymal saturated phosphatidylcholine lung pools and biologic half-lives of 100 to 360 hours for parenchymal saturated phosphatidylcholine estimated previously in 29-day spontaneously breathing rabbits in the newborn period.[7] Therefore

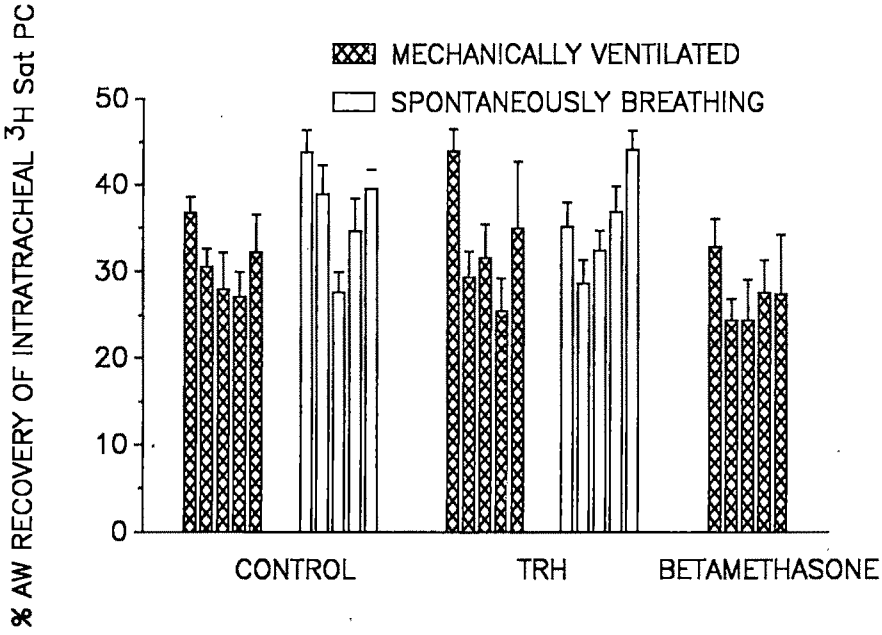


Fig. 5. Alveolar wash *(AW)* recovery of tritium-labeled rabbit surfactant given intratracheally at birth. These recoveries, expressed as percentages of radioactivity administered at birth, resulted from combined effects of surfactant saturated phosphatidylcholine *(Sat PC)* clearance, lung uptake, and resecretion into lungs. Rapid mobilization and resecretion of saturated phosphatidylcholine pools from lung parenchyma appeared to be more rapid than combined rates of lung uptake and alveolar degradation at the later time points. *Bars* from left to right within each treatment group again represent group means ± SE for rabbits killed at 1, 4, 8, 15 and 24 hours.

the large increases seen postnatally in saturated phosphatidylcholine alveolar pools after the onset of ventilation were much more dependent on changes in secretion and recycling resulting in mobilization of existing pools in the lung. Neither mechanical ventilation nor maternal hormonal treatments with TRH or corticosteroids resulted in decreasing the relative importance of mobilizing existing pools.

Because there was very little degradation of saturated phosphatidylcholine after birth, we were able to estimate net secretion into the air spaces by measuring the percentages of ^{14}C and ^{32}P incorporated from precursors into saturated phosphatidylcholine that were subsequently recovered from the alveolar washes. Assuming a totally efficient alveolar wash and negligible degradation, the net secretion would be equal to the total amount of radiolabel secreted into the air spaces minus the total amount reassociated with the lung. The percentages of precursor-labeled saturated phosphatidylcholine recovered from the alveolar washes would be directly proportional to the net secretion, assuming that the fractions of saturated phosphatidylcholine in the lung that were not part of surfactant pools remained constant. This assumption appeared to be valid because we saw no effects of antenatal maternal treatments with TRH or corticosteroids on either the pool sizes of saturated phosphatidylcholine recovered from the alveolar space or the estimated net secretion into the alveolar space. Although this study was designed primarily to examine the effects of antenatal maternal treatments with TRH or corticosteroids, the most intriguing differences were between those rabbits allowed to spontaneously breathe and those that were mechanically ventilated whether receiving antenatal TRH or vehicle. Preterm rabbits allowed to spontaneously breathe had significantly higher net secretions of saturated phosphatidylcholine at 15 and 24 hours compared with those that were mechanically ventilated. Spontaneously breathing newborns also had larger increases in their alveolar pools at 24 hours compared with those mechanically ventilated, also consistent with higher net secretions of saturated phosphatidylcholine because clearance and synthesis were not altered by the ventilatory style. Mechanically ventilated rabbits were given the same amount of oxygen, antibiotics, and nutrition as the preterm rabbits allowed to spontaneously breathe; there were no significant differences in the blood gases for those in which the higher net secretions were seen at 15 and 24 hours. We could not separate the effects of anesthesia and tracheostomy from those of mechanical ventilation in this study, but the slowing of net secretion appeared to be a secondary effect of lung injury or other changes that occurred in the lung after more prolonged ventilation because there were no differences seen at the earlier time points.

In summary, this was the first study where preterm

rabbits have been mechanically ventilated for up to 24 hours, and the majority tolerated the procedure well as assessed by terminal blood gas analysis. The 24-hour postnatal course of the preterm rabbit at 29 days' gestation provided a unique opportunity to observe a period of extremely rapid mobilization of lung-associated surfactant saturated phosphatidylcholine into the alveolar space. Even tritium-labeled surfactant saturated phosphatidylcholine given intratracheally failed to continuously decrease in the alveolar space with time (with a tendency to actually increase from 8 to 24 hours), suggesting that recycling and resecretion became as fast or faster than uptake by the lung. Antenatal maternal TRH or betamethasone in doses that improved lung function in 27 to 29-day preterm rabbits did not affect saturated phosphatidylcholine synthesis, clearance, or net secretion postnatally at 29 days' gestation. Mechanical ventilation was associated with a slower mobilization of lung-associated saturated phosphatidylcholine stores into the alveolar space after birth. Our results suggest that the ventilated preterm rabbit model may also be useful for studying relationships between mechanical ventilation and lung injury.

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Nω-Nitro-L-arginine, an inhibitor of nitric oxide synthesis, increases blood pressure in rats and reverses the pregnancy-induced refractoriness to vasopressor agents

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OBJECTIVE: With Nω-nitro-L-arginine, a potent inhibitor of nitric oxide synthesis, we tested the hypothesis that nitric oxide plays a functional role in the blunted pressor responsiveness seen during pregnancy.
STUDY DESIGN: A group of six pregnant rats were instrumented on the fourteenth day of gestation and studied on days 19 and 20, as well as 7 days post partum. Another group of six virgin rats were similarly prepared and used 5 days after surgery. Blood pressure and heart rate were monitored in conscious freely moving animals before and during the administration of drugs or placebo. Results were analyzed, by one-way repeated-measures analysis of variance, with Dunnett's *t* test, or by paired *t* test where applicable.
RESULTS: Basal mean arterial pressure and heart rate were 90.8 ± 3.0 mm Hg and 330 ± 6 beats/min in pregnant animals and 107.1 ± 3.2 mm Hg and 315 ± 7 beats/min in nonpregnant animals. Pressor responses to angiotensin II, vasopressin, and norepinephrine were attenuated in gravid animals. Infusion of Nω-nitro-L-arginine significantly and in a dose-dependent manner increased mean arterial pressure and reduced heart rate. These effects could be completely reversed by L-arginine administration. Changes in mean arterial pressure were higher during pregnancy as compared with postpartum values. Nω-nitro-L-arginine infusion potentiated pressor responses to all three vasopressors, resulting in dose-response curves that were significantly shifted to the left, making them virtually identical in pregnant and postpartum rats.
CONCLUSION: Our data support the emerging view that nitric oxide plays a key role in the regulation of blood pressure during pregnancy. (Am J Obstet Gynecol 1992;166:1560-7.)

Key words: Rat, pregnancy, nitric oxide, Nω-nitro-L-arginine, pressor responsiveness

Attenuated responses to vasopressor agents and reduced peripheral resistance and blood pressure are among the hemodynamic changes that characterize pregnancy. The mechanism(s) responsible for these phenomena is not known. Possible changes in affinity and/or density of vasoconstrictor receptors as causative factors have been ruled out.[1,2] In early studies inhibitors of cyclooxygenase (the enzyme responsible for the conversion of arachidonic acid to prostanoids) reduced the dose of vasopressors required to raise blood pressure in pregnant women; therefore it was proposed that vasodilator prostaglandins are responsible for the pregnancy-associated circulatory changes.[2-4] However, subsequent studies in the rat and guinea pig failed to corroborate these observations,[5,6] and the prostaglandin theory remains controversial. There is growing evidence that endothelium-derived factors may play important, local paracrine functions in the regulation of vascular tone.[7] We[8] have reported recently that, whereas pressor responses to angiotensin II and vasopressin were typically attenuated in chronically instrumented, conscious pregnant rats, the endothelin ET-1, a highly potent endothelium-derived vasopressor peptide, was equally effective in raising mean arterial pressure (MAP) in both pregnant and nonpregnant animals. Endothelium-derived relaxing factor, which is identical in its action to nitric oxide,[9] is synthesized by endothelial cells from the guanidino group of L-arginine.[10] Moreover, certain analogs of arginine, such as N-monomethyl-L-arginine and Nω-nitro-L-arginine, have been found to inhibit nitric oxide synthesis and vasodilatation in vitro,[11,12] as well as raise blood pressure in vivo.[11,13] We made use of the latter inhibitor, which has been reported to be significantly more potent than N-monomethyl-L-arginine,[11,12] to examine the possible role of endothelium-derived relaxing factor–nitric oxide in pregnancy-attenuated basal MAP and MAP responses to angiotensin II, vasopressin, and norepinephrine in chronically instrumented rats during preg-

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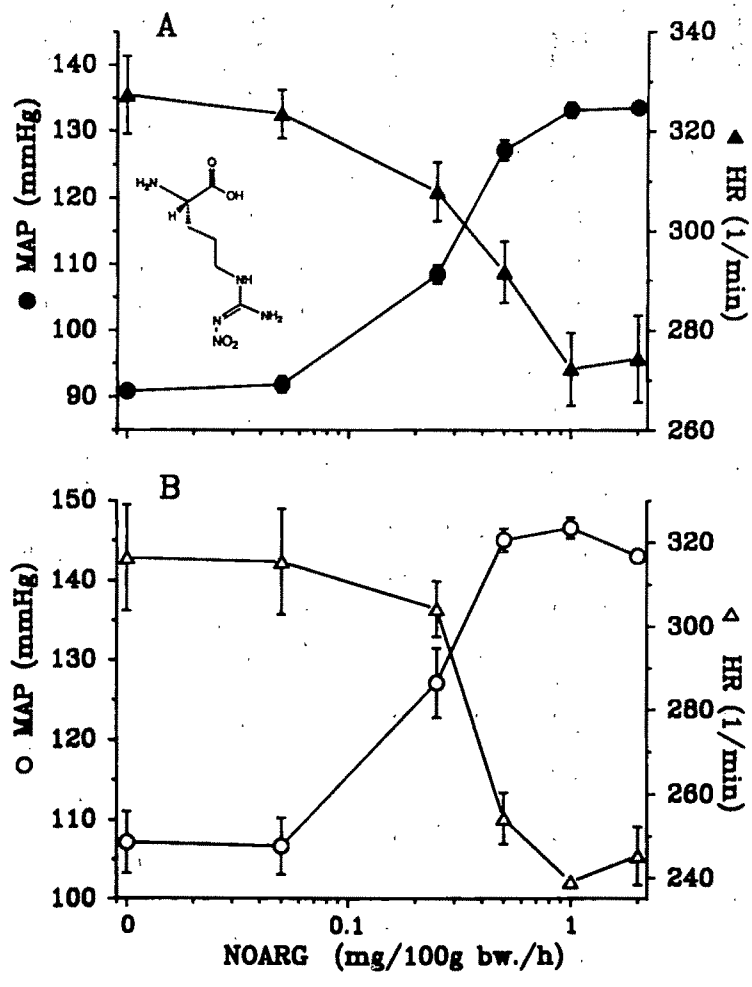


Fig. 1. Effect of infusion of Nω-nitro-L-arginine *(NOARG)* on MAP and heart rate in pregnant **(A)** and virgin **(B)** rats. *Inset,* Molecular structure of Nω-nitro-L-arginine. Experimental conditions are detailed in Material and Methods section.

nancy and 7 days post partum. Our results support the notion that blunted pressor responsiveness during normal gestation is due largely to increased elaboration of endothelium-derived–nitric oxide.

Material and methods

Reagents. Synthetic angiotensin II was purchased from Calbiochem (La Jolla, Calif.). Nω-nitro-L-arginine methyl ester, arginine vasopressin, norepinephrine, and all other chemicals used were obtained from Sigma Chemical Co. (St. Louis).

Preparation of chronically catheterized rats. Wistar-derived pregnant rats (LATI, Gödöllö, Hungary) weighing 260 to 300 gm were kept in standard metabolic cages for 3 days before surgery. The guidelines approved by the animal research committee of Semmelweis Medical University for the care and use of animals were followed throughout this study. Animals were instrumented while they were under surgical level anesthesia on the fourteenth day of gestation (delivery on day 21 or 22) according to Fejes-Tóth et al.[14] In brief, with the animals under ketamine (75 mg/kg body weight) and pentobarbital (15 mg/kg body weight) anesthesia, polyethylene catheters (PE-10, Clay Adams, Parsippany, N.J.) were implanted into the abdominal aorta and vena cava through the ventral tail artery and femoral vein, respectively. The venous catheter was guided subcutaneously to the root of the tail. Both catheters emerging from the tail and the wounds were protected by an acrylic cuff glued to the tail. The cuff was connected to a stainless steel helical spring leading through the top of the metabolic cage, allowing easy hookup to recorder and infusion pump.

Measurement of MAP and pressor responsiveness. On day 19 sterile saline solution was infused at 0.5 ml/100 gm body weight per hour via the venous catheter, and MAP was recorded in conscious, freely moving rats by means of an electromanometer (Medicor, Budapest) and a Statham P23dP pressure transducer (Hato Rey, Puerto Rico). After an equilibration period

Table I. Effect of infusion of L-arginine and combined infusion of L-arginine with Nω-nitro-L-arginine on angiotensin II–, vasopressin-, and norepinephrine-induced pressor responsiveness in virgin and pregnant rats

	L-Arginine (5 mg/100 gm body weight/hr)			
Group	*Saline solution*	*Angiotensin II*	*Vasopressin*	*Norepinephrine*
Virgin MAP (mm Hg)	110.0 ± 4.1	144.3 ± 2.9	140.1 ± 2.5	146.5 ± 2.5
Pregnant MAP (mm Hg)	92.3 ± 3.9	115.8 ± 2.1	107.8 ± 4.5	110.3 ± 3.4

Chronically instrumented rats were infused at 0.5 ml/100 gm body weight via venous catheter with L-arginine (5 mg/100 gm body weight) or with L-arginine (5 mg/100 gm body weight) + Nω-nitro-L-arginine (1 mg/100 gm body weight) simultaneously. After an equilibration period of 1 hour, a single dose of angiotensin II (10 pmol/100 gm body weight), vasopressin (7 pmol/100 gm body weight), or norepinephrine (60 pmol/100 gm body weight) was injected into the vena cava and changes in MAP were monitored continuously as described in Material and Methods.

of 2 hours, each animal received increasing doses of angiotensin II in a volume of 5 μl/100 gm body weight, via the catheter implanted into the vena cava, followed by an injection of 100 μl of saline solution. A period of 20 minutes was allowed to elapse between the administration of incremental doses. The same procedure was repeated with vasopressin and norepinephrine. The next day Nω-nitro-L-arginine, dissolved in sterile saline solution, was infused at 1 mg/100 gm body weight per hour in a volume of 0.5 ml/hr, and MAP and pressor responses to angiotensin II, vasopressin, and norepinephrine were recorded as on the previous day. Pups were removed immediately after delivery, and the experiments were repeated on the same animals 7 days post partum or on weight- and age-matched virgin rats.

Measurement of effects of Nω-nitro-L-arginine in pregnant and virgin rats. Day 14 pregnant rats ($n = 6$) and weight- and time-matched virgin rats ($n = 6$) were instrumented and kept as described. Five days later sterile saline solution was infused at 0.5 ml/100 gm body weight via venous catheter. After an equilibration period of 1 hour increasing doses of Nω-nitro-L-arginine (0.05 to 2 mg/100 gm body weight) were infused for 1 hour at a rate of 0.5 ml/hr, and MAP and heart rate were recorded continuously in conscious, freely moving animals.

Statistical analysis. Pressor responses are expressed as MAP changes from preinfusion baseline (mean ± SEM). Statistical evaluation of dose-response values was done by one-way repeated-measures analysis of variance and Dunnett's t test. Comparisons of saline solution and Nω-nitro-L-arginine treatment and of pregnancy and postpartum values were done by paired t test.

Results

Basal MAP was significantly higher ($p < 0.005$) in nonpregnant animals (107.1 ± 3.2 mm Hg) as compared with pregnant values (90.8 ± 3.0 mm Hg). Infusion of Nω-nitro-L-arginine significantly and in a dose-dependent manner increased MAP and reduced heart rate in both pregnant and virgin animals, with an identical median effective dose of 0.25 ± 0.01 mg/100 gm body weight (Fig. 1). The maximal MAP responses were higher, though not significantly so ($p = 0.091$), in pregnant animals (42.7 ± 1.04 mm Hg) as compared with virgin animals (36.0 ± 3.6 mm Hg). Changes in heart rate were higher, but again not significantly so ($p = 0.093$), in virgin rats (77.3 ± 13.5 × 1/min) versus pregnant rats (55.0 ± 4.7 × 1/min). However, when Nω-nitro-L-arginine (1 mg/100 gm body weight per hour)–induced increments in MAP in late-pregnant animals (44.0 ± 3.4 mm Hg) were compared with those obtained in the same rats 7 days post partum (33.8 ± 7.6 mm Hg), these were found to be significantly different ($p < 0.005$ by paired t test). Nevertheless, MAP remained significantly ($p < 0.01$) higher in nonpregnant animals during the Nω-nitro-L-arginine infusion (Fig. 2). Infusion of a fivefold excess of L-arginine completely reversed Nω-nitro-L-arginine–induced pressor effects and reflex bradycardia (Fig. 3). Administration of L-arginine alone did not alter MAP in saline solution–treated rats and did not reduce the pressor responsiveness of angiotensin II, vasopressin, or norepinephrine. In animals pretreated with L-arginine (5 mg/100 gm body weight per hour), Nω-nitro-L-arginine (1 mg/100 gm body weight per hour) did not influence MAP or heart rate and did not affect angiotensin II-, vasopressin-, and norepinephrine-induced pressor responses (Table I).

Angiotensin II, vasopressin, and norepinephrine caused a dose-dependent rise in MAP. Dose-response curves for all agonists were shifted significantly to the right in pregnant animals (Figs. 4, 5, and 6). Angiotensin II and vasopressin were more potent vaso-

L-Arginine (5 mg/100 gm body weight/hr) plus Nω-nitro-L-arginine (1 mg/100 gm body weight/hr)			
Saline solution	*Angiotensin II*	*Vasopressin*	*Norepinephrine*
108.9 ± 3.9	140.3 ± 3.5	139.9 ± 4.4	142.7 ± 3.9
91.2 ± 4.1	120.0 ± 6.7	110.1 ± 3.6	95.3 ± 5.4

constrictors than norepinephrine. The angiotensin II–generated response curve yielded median effective dose values of 6.7 ± 0.69 and 12.5 ± 2.29 pmol/100 gm body weight for the nonpregnant and pregnant animals, respectively. The corresponding median effective dose values were 5.6 ± 1.79 and 10.2 ± 2.10 pmol/100 gm body weight for vasopressin and 43.4 ± 4.91 and 105.9 ± 8.41 pmol/100 gm body weight for norepinephrine. The maximal changes in MAP evoked by all three agonists were not significantly different. Infusion of Nω-nitro-L-arginine potentiated pressor responses to the pressor agent, resulting in dose-response curves that were significantly shifted to the left. Importantly, Nω-nitro-L-arginine reversed the blunted pressor responses to all agonists obtained during gestation, yielding dose-response curves that were virtually identical to those generated in nonpregnant rats (Figs. 4, 5, and 6). Median effective dose values for angiotensin II, vasopressin, and norepinephrine were reduced to 1.0 ± 0.18, 3.1 ± 1.08, and 17.3 ± 5.17 pmol/100 gm body weight, respectively. However, the maximal changes of MAP were not significantly different in either Nω-nitro-L-arginine or control (saline solution–treated) groups. The dose-response curves of all agonists used in this study were superimposable in virgin rats and in rats 7 days post partum (data not shown).

Comment

In the current study with chronically instrumented conscious rats, we have been able to show that intravenous infusion of Nω-nitro-L-arginine, a potent inhibitor of nitric oxide synthesis,[11, 12] causes a significant increase in basal MAP in nonpregnant rats but particularly in gravid rats. More importantly, during continuous infusion of Nω-nitro-L-arginine, the blunted vasopressor response to several agonists, which is characteristic of human and rodent pregnancy,[15-17] was obliterated.

Endothelium-derived relaxing factor is believed to be either identical or very similar to nitric oxide, and it contains a reactive nitrogen moiety synthesized from L-arginine as an endogenous substrate in vascular endothelium and in other cells.[10, 18, 19] Structural analogs of L-arginine, such as Nω-nitro-L-arginine or N-monomethyl-L-arginine, competitively inhibit the formation of nitric oxide, which can be reversed by excess amounts of L-arginine.[20] Indeed, the current study has demonstrated that in the presence of a continuous infusion of a fivefold excess of L-arginine, Nω-nitro-L-arginine had no influence on basal blood pressure or on the pressor effects of angiotensin II, vasopressin, or norepinephrine, providing strong evidence that its action is due specifically to the inhibition of nitric oxide synthesis from L-arginine. Additional evidence that the effect of L-arginine is specific was obtained by showing that the basal blood pressure and the pressor responses to angiotensin II, vasopressin, and norepinephrine were not affected by L-arginine treatment alone.

Normal human pregnancy is accompanied by striking cardiovascular changes. There is an increase in heart rate, cardiac output, and blood volume and a decrease in arterial pressure and responsiveness to angiotensin II.[16] The chronically instrumented conscious rat has been shown to exhibit gestation-induced cardiovascular and renal changes very similar to those observed in normal pregnant women,[5, 21] rendering it an appropriate and useful model for the investigation of pregnancy-induced hemodynamic changes, including the attenuated responsiveness to pressor agents.[22] These changes have been attributed to increased production of vasodilator prostanoids.[2-4] However, this concept has been disputed since inhibitors of cyclooxygenase failed to alter pressor responsiveness in the rat[5] and guinea pig.[6] There is growing evidence that substances derived from the endothelium may play crucial roles in the local paracrine regulation of blood pressure. Supporting the role of endothelium-derived relaxing factor in blood pressure regulation in the gravid female are the findings of an increased relaxant effect of acetylcholine in vascular preparations with intact endothelium of pregnant guinea pig,[23] as well as the significant increase during gestation in plasma and urinary levels of cyclic guanosine monophosphate,[24] be-

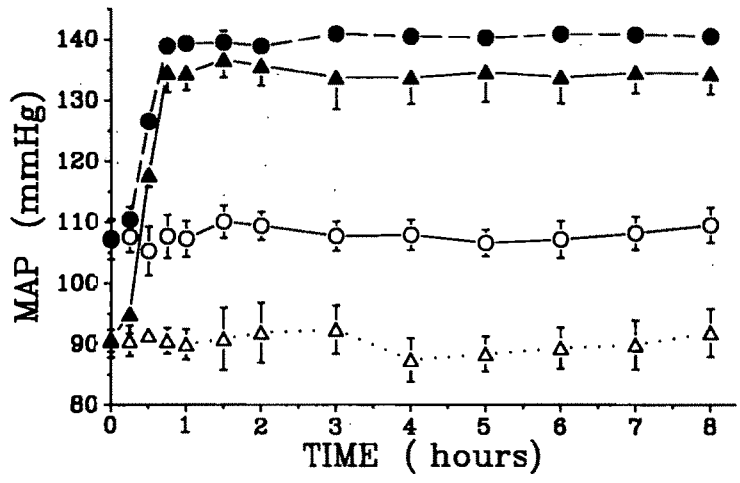


Fig. 2. MAP changes during infusion of Nω-nitro-L-arginine at rate of 1 mg/100 gm body weight per hour and saline solution, both at rate of 0.5 ml/hr, in chronically instrumented, conscious, pregnant and nonpregnant rats. Saline solution and Nω-nitro-L-arginine infusions were started at time 0. Values are mean ± SEM ($n = 6$). *Open symbols,* MAP measured during saline solution infusion in rats during pregnancy (△) and 7 days post partum (○). Every *point* of nonpregnant animals is significantly ($p < 0.005$) higher than corresponding pregnant values. *Closed symbols,* MAP values obtained during Nω-nitro-L-arginine infusion in pregnant (▲) and nonpregnant (●) animals. MAP of Nω-nitro-L-arginine–infused nonpregnant rats is significantly ($p < 0.05$) higher than corresponding pregnant values.

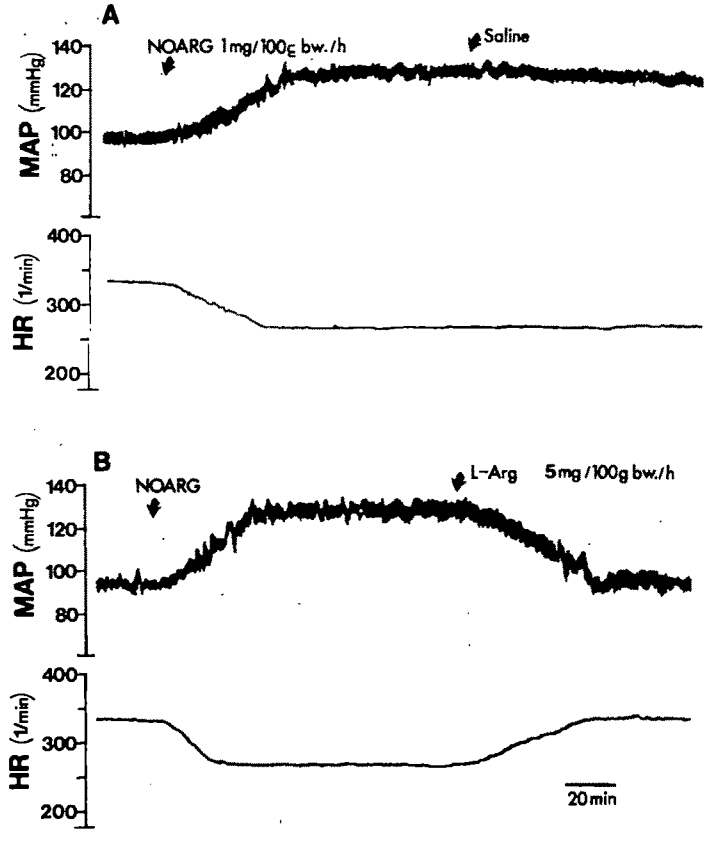


Fig. 3. L-Arginine reversed Nω-nitro-L-arginine–induced vasoconstriction. Chronically catheterized pregnant rats were infused with Nω-nitro-L-arginine (1 mg/100 gm body weight per hour) at rate of 0.5 ml/100 gm body weight per hour. After 2 hours infusion was continued with saline solution **(A)** or L-arginine (5 mg/100 gm body weight per hour) **(B)** at same flow rate. MAP and heart rate were monitored continuously, as described in Material and Methods.

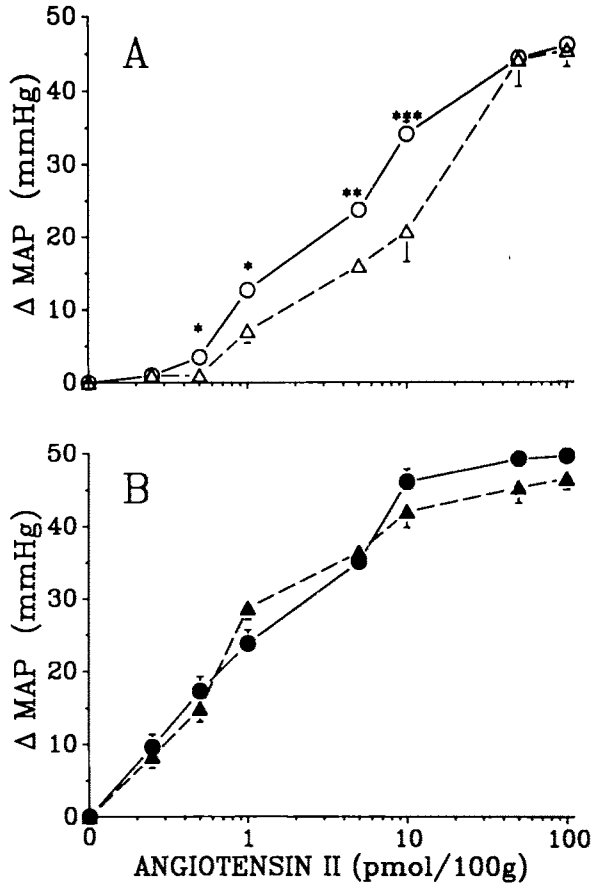


Fig. 4. Pressor responses to angiotensin II in pregnant (△, ▲) and nonpregnant (○, ●) rats during saline solution **(A)** and Nω-nitro-L-arginine **(B)** infusion. Values are mean ± SEM ($n = 6$) of changes in MAP. *One asterisk*, $p < 0.05$; *two asterisks*, $p < 0.01$; *three asterisks*, $p < 0.005$; all comparisons are with pregnant values.

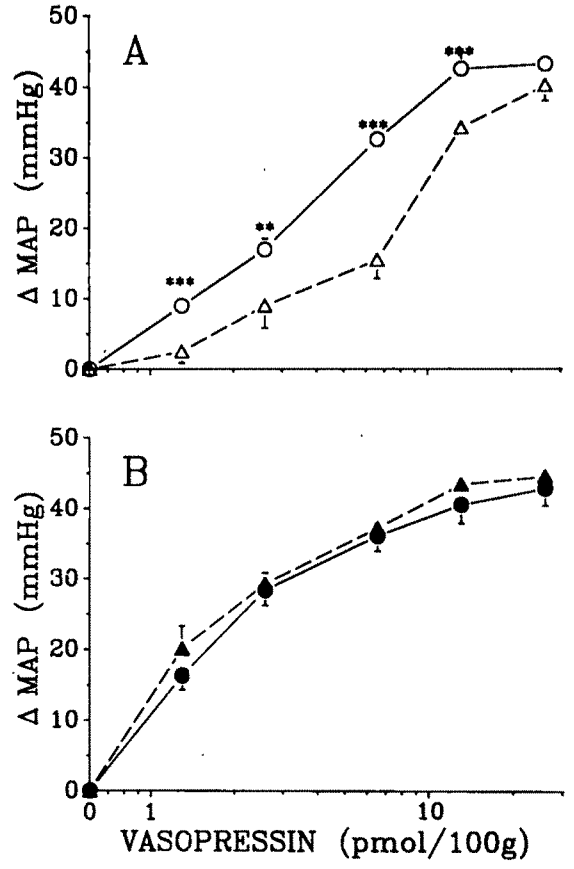


Fig. 5. Pressor responses to vasopressin in pregnant (△, ▲) and nonpregnant (○, ●) rats during saline solution **(A)** and Nω-nitro-L-arginine **(B)** infusion. Values are mean ± SEM ($n = 6$) of changes in MAP. *Two asterisks*, $p < 0.01$; *three asterisks*, $p < 0.005$; all comparisons are with pregnant values.

lieved to be the intracellular mediator of endothelium-dependent vasorelaxation.[7] It remains to be clarified whether pregnancy-induced vasodilatation is due chiefly to increased elaboration of nitric oxide by the vascular endothelium or also to an increase in the activity of the nitric oxide–sensitive guanylate cyclase of vascular smooth muscle cells.

It may be relevant to point out that the loss of refractoriness to pressor agents is the hallmark of pregnancy-associated hypertension.[15] Myatt et al.[25] have recently presented evidence that basal release of nitric oxide contributes to maintenance of vascular tone in placental circulation, whereas blockade of prostaglandin synthesis by meclofenamate had no effect on the flow pressure of the placental circulation.[25] Further, an impairment in endothelium-derived relaxing factor release has been observed in vitro in vessels of spontaneously[26] and experimentally hypertensive[27] animals. In the anesthetized rabbit the blockage of endothelium-derived relaxing factor production by N-monomethyl-L-arginine caused a long-lasting arterial blood pressure increase, suggesting that a reduction in the synthesis of nitric oxide may contribute to the pathogenesis of hypertension.[28] Pinto et al.[29] recently found a significantly lower release of endothelium-derived relaxing factor by the umbilical arteries and veins collected from patients with pregnancy-induced hypertension compared with tissues from normal subjects, supporting the hypothesis that impaired endothelium-derived relaxing factor synthesis may be an important factor in the etiology of pregnancy-induced hypertension.

Even though the cyclooxygenase inhibitor indomethacin failed to restore the blunted systemic and renal responsiveness in instrumented, conscious, late-gravid rats,[5] a modulating role for prostacyclin, possibly by interacting in synergistic fashion with endothelium-derived relaxing factor–nitric oxide, as has been demonstrated with respect to platelet aggregation,[30] should not be ruled out. Ahokas and Sibai[31] reported that administration of N-monomethyl-L-arginine to spontaneously hypertensive pregnant rats raised blood pres-

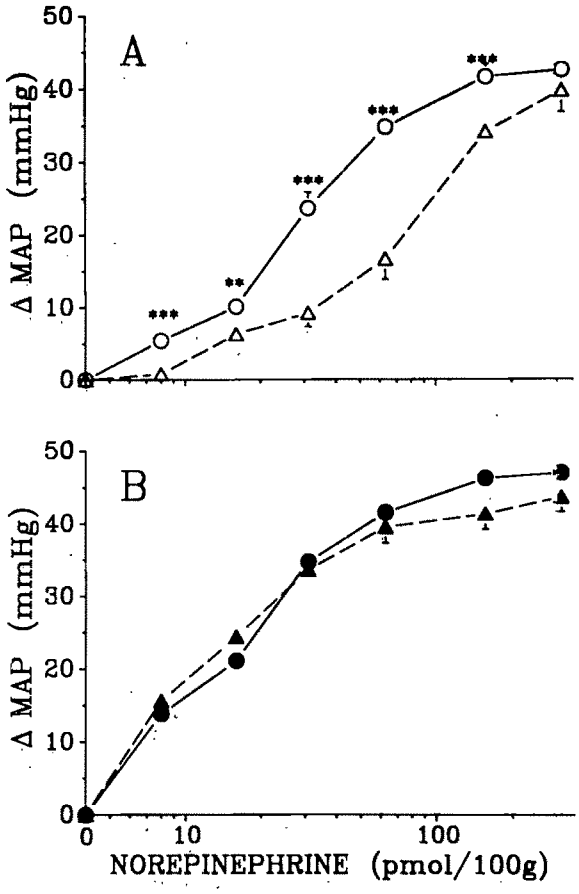


Fig. 6. Pressor responses to norepinephrine in pregnant (△, ▲) and nonpregnant (○, ●) rats during saline solution (*A*) and Nω-nitro-L-arginine (*B*) infusion. Values are mean ± SEM ($n = 6$) of changes in MAP. *Two asterisks*, $p < 0.01$; *three asterisks*, $p < 0.005$; all comparisons are with pregnant values.

sure and blunted pressor responses to angiotensin II; these parameters were unaffected by pretreatment with indomethacin. However, N-monomethyl-L-arginine failed to significantly affect pressor responses to angiotensin II in pregnant normotensive WKY rats. In a recent study Umans et al.[32] also observed a significant increase in MAP of conscious, instrumented rats in response to a single intravenous bolus of N-monomethyl-L-arginine, a less-potent inhibitor of nitric oxide synthesis. However, these investigators failed to detect the blunted pressor responsiveness occurring during normal gestation and failed to detect an increased responsiveness to N-monomethyl-L-arginine in pregnant versus virgin rats. The model used in the current study offers the advantage of comparing the same groups of animals under identical experimental treatments during late gestation and 7 days post partum. This and the choice of inhibitors may account for the apparent discrepancy.

Taken together, the current results and those of other investigators[23-25, 29] provide strong suggestive evidence that endothelium-derived factors play key roles in the regulation of blood pressure in the gravid female to meet the requirements of the changing internal environment of pregnancy.

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Role of prostaglandins in the metabolic responses of the fetus to hypoxia

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OBJECTIVE: The effect of inhibiting prostaglandin synthesis on the fetal metabolic response to hypoxemia was examined by infusing indomethacin during periods of reduced maternal uterine blood flow.

STUDY DESIGN: In seven fetal sheep we administered a 6-hour infusion of either indomethacin ($n = 5$), indomethacin plus prostaglandin E_2, or a vehicle solution ($n = 5$). The last 4 hours of each infusion period coincided with a period of fetal hypoxemia induced by reduced maternal uterine blood flow.

RESULTS: During reduced maternal uterine blood flow indomethacin infusions caused a significantly greater reduction in pH_A (reduced from 7.36 ± 0.01 to 7.10 ± 0.02) than both the vehicle (from 7.36 ± 0.01 to 7.20 ± 0.03) and indomethacin plus prostaglandin E_2 infusions (from 7.36 ± 0.01 to 7.18 ± 0.02). Before reduced maternal uterine blood flow was induced, indomethacin significantly elevated fetal plasma glucose and lactate concentrations from 0.6 ± 0.04 and 2.2 ± 0.1 to 1.3 ± 0.2 and 6.7 ± 0.7 mmol/L, respectively. During reduced maternal uterine blood flow indomethacin caused a significantly greater increase in plasma glucose and lactate concentrations than the vehicle; plasma glucose and lactate concentrations increased to a maximum of 1.8 ± 0.2 and 22.7 ± 0.8 mmol/L, respectively, during indomethacin infusions compared with 1.1 ± 0.1 and 15.7 ± 1.7 mmol/L, respectively, during vehicle infusions. The addition of prostaglandin E_2 to the indomethacin infusion prevented the enhanced increase in glucose and lactate concentrations during reduced maternal uterine blood flow and caused a significant increase in fetal plasma insulin concentrations from 12.6 ± 0.7 to 60.9 ± 28.1 μU/ml.

CONCLUSION: The inhibition of prostaglandin synthesis during fetal hypoxemia alters the metabolic response of the fetus, leading to a severe metabolic acidosis. (Am J Obstet Gynecol 1992;166:1568-75.)

Key words: Fetus, hypoxia, asphyxia, indomethacin, glucose, lactate, insulin

Fetal hypoxia is a common cause of fetal morbidity and mortality and can result from a number of causes, including umbilical cord occlusion, maternal cigarette smoking, and reduced uteroplacental circulation. The blood gas and pH changes and many of the endocrine, biophysical, and cardiovascular responses that occur in the fetus during acute periods of hypoxemia are not sustained if the hypoxemia is prolonged.[1-4] In particular the fetal arterial acidosis, which develops initially after reducing maternal uterine blood flow, is corrected after 12 to 16 hours.[3,4] We have suggested that this correction in fetal blood pH results from a metabolic adaptation to the hypoxemia[4]; our study is directed toward an understanding of the mechanisms involved in this adaptation.

Prostaglandin E_2 (PGE_2) is considered to be an important circulating fetal hormone released by the placenta.[5-7] Fetal plasma PGE_2 concentrations gradually increase in an exponential-like manner toward term[7] and can be precociously elevated by fetal hypoxemia,[4] hypoglycemia,[7,8] and hyperthermia.[9] In addition, PGE_2 is thought to play an important metabolic role in the fetus by regulating insulin release,[10] and PGE_2 has recently been identified as the placental factor that prevents catecholamine-mediated brown fat metabolism in the fetus.[11]

In a previous study we suggested that correction of fetal arterial acidosis, which occurred during prolonged hypoxia, primarily resulted from an increase in the uptake and metabolism of glucose and lactate by the fetus.[4] Because we also observed that the increase in fetal arterial pH during prolonged hypoxemia closely followed the increase in fetal plasma PGE_2 concentrations, the aim of the present study was to determine the role of PGE_2 in the fetal metabolic adaptation to hypoxemia. In particular we wished to determine the effect of inhibiting prostaglandin release during fetal hypoxemia on fetal plasma glucose, lactate, and insulin concentrations. We hypothesized that the gradual increase in circulating PGE_2 concentrations in the fetus

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during hypoxemia plays an important metabolic role by reducing fetal blood glucose and, hence, lactate concentrations.

Material and methods

Animal preparation. Surgery was performed under aseptic conditions on seven Border Leicester × Merino ewes between 110 and 117 days of gestation (term is 145 days). Anesthesia was induced with 5% thiopental sodium in water (intravenously) and was maintained after tracheal intubation with 1.5% halothane (oxygen/nitrous oxide, 50:50 vol/vol). In all ewes polyvinyl catheters (SV116, Dural Plastics, Sydney, Australia) were implanted into the maternal jugular vein and amniotic cavity. An adjustable clamp[12] was placed around the maternal common internal iliac artery to produce controlled reductions in maternal uterine blood flow.[13, 14]

In each fetus polyvinyl catheters (SV65, Dural Plastics) were implanted into the carotid artery and jugular vein. A polyvinyl catheter (SV116) was also implanted into the trachea of the fetus to detect fetal breathing movements. Antibiotics (2 ml Streptopen intramuscularly, Glaxovet, Australia) were administered to each fetus before the uterine incision was closed. Fetal catheters and the control cable for the vascular clamp were exteriorized through an incision in the right flank of the ewe. The ewes were allowed to recover for at least 5 days after surgery before experimentation.

Experimental protocol. Three separate 8-hour experiments were performed, each consisting of a 6-hour infusion period and a 2-hour recovery period. The last 4 hours of the 6-hour intravenous infusion corresponded to a period of moderate fetal asphyxia induced by reducing maternal uterine blood flow.[14] The three experiments differed by the type of infusion administered to the fetus: (a) a vehicle solution, (b) indomethacin (17 gm/min), or (c) indomethacin (17 gm/min) plus PGE_2 (2 gm/min). Fetal arterial blood samples were collected before the infusion (−2 hours), immediately before the period of reduced maternal uterine blood flow (0 hours), and at 0.5, 1, 2, 4, and 6 hours after the onset of reduced maternal uterine blood flow for the measurement of Pa_{O_2}, Pa_{CO_2}, pH_A, fetal arterial blood oxygen saturation (Sa_{O_2}), and glucose and lactate concentrations. Fetal arterial blood samples were also collected for the measurement of insulin concentrations at −2, 0, 2, and 4 hours. Fetuses were allowed to recover for ≤5 days between experiments.

Fetal Pa_{O_2}, Pa_{CO_2}, and pH_A were measured with a blood gas and acid base analyzer (ABL30, Radiometer, Denmark); fetal Sa_{O_2} was measured with a hemoximeter (OSM2, Radiometer). Fetal arterial pressure and heart rate were recorded continuously on a polygraph (7D, Grass) throughout each experiment. Arterial pressures were recorded after subtraction of amniotic fluid pressure. These experiments adhere to the Monash University guidelines on the care and use of experimental animals.

Insulin assay. Fetal plasma insulin concentrations were measured with a human insulin radioimmunoassay kit (code IM.78, Amersham, United Kingdom) modified for ovine fetal plasma. The antisera to human insulin, human insulin standards (range 0 to 320 μU/ml), iodine 125–labeled insulin, and assay buffers were provided with the kit. The manufacturer's protocol was used except for the following modifications: to increase the sensitivity of the assay, the concentration of antisera and ^{125}I-labeled insulin was one quarter and one half, respectively, of the concentration recommended; the assay was incubated overnight at 4° C, and bound and free insulin was separated with polyethylene glycol 6000 (10%; 0.03% sodium azide). All assay tubes were centrifuged at 2500 rpm for 20 minutes at 4° C (Beckman J6B) before the supernatant was aspirated off. The radioactivity remaining in the pellet was quantified using a gamma counter (Crystal 5400, Packard, USA). The intraassay coefficient of variation was 11.1% ($n = 16$) at an insulin concentration of 72.8 ± 2.0 μU/ml of fetal plasma. No significant deviation from parallelism ($p > 0.1$) was found between a standard curve in buffer, a standard curve in plasma, and different volumes of a single plasma sample. A significant ($p < 0.005$) correlation was found between the amount of insulin added to plasma and the amount measured by the assay ($y = 0.98x + 0.58$, $r = 0.998$).

Plasma PGE_2 glucose and lactate concentrations. Fetal plasma PGE_2 concentrations were measured by radioimmunoassay with antisera and procedures previously described.[7] Fetal plasma glucose concentrations were measured enzymatically by a previously described method.[15] Fetal plasma lactate concentrations were also measured enzymatically with a glucose-lactate analyzer (2300GL, Yellow Springs Instruments).

Statistical analysis. The results are presented as mean ± SEM. Data on fetal blood gases and pH_A and fetal plasma, PGE_2, glucose, lactate, and insulin concentrations (after log transformation) were analyzed by a three-way analysis of variance with treatment (type of infusion), time, and animals as factors. All three experiments were completed in only four of the seven animals studied. Analyses of variance were performed only with these four fetuses. If a significant effect of treatment was found between any of the treatments, the significant treatment was analyzed separately by a two-way analysis of variance with animals and time as factors. This analysis was followed by a Student–Newman-Keuls multiple-range test for differences between time periods. Differences between treatments for a

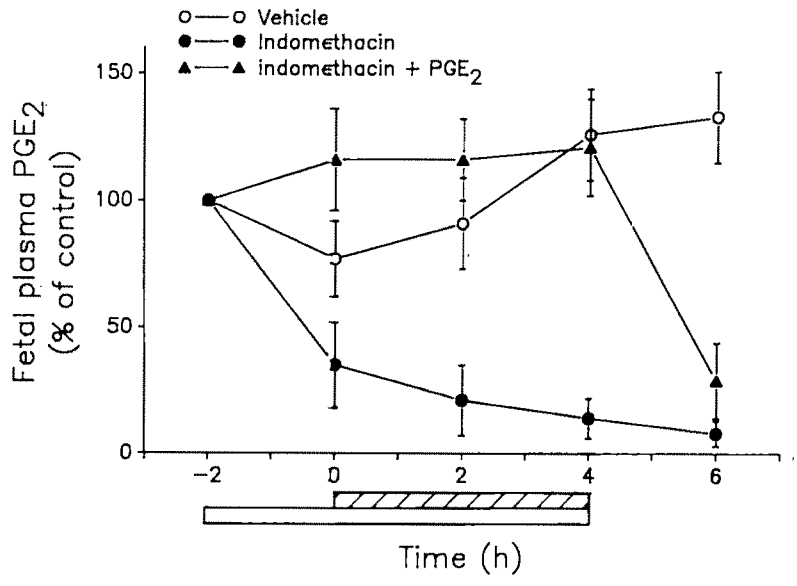


Fig. 1. Fetal plasma PGE_2 concentrations expressed as a percentage of preinfusion control values during either a 6-hour vehicle *(open circles)*, indomethacin *(solid circles)*, or indomethacin plus PGE_2 *(triangles)* infusion. The period of reduced maternal uterine blood flow *(hatched bar)* coincided with the last 4 hours of the 6-hour infusions *(open bar)*.

given time period were analyzed by a nonpaired Student's t test. Unless otherwise stated, the level of significance reported in the text is $p < 0.05$.

Results

Fate of fetuses. All fetuses survived to or near term (140.1 ± 0.8 days), at which time the ewes and fetuses were killed by an overdose of pentobarbital sodium All fetuses were classified as being healthy at the start of each experiment as judged by their blood gas and acid-base status.

Fetal plasma PGE_2 concentrations. The vehicle infusion alone did not significantly affect fetal plasma PGE_2 concentrations, whereas inducing reduced maternal uterine blood flow during the vehicle infusion caused an increase in PGE_2 concentrations from a mean control value of 4.3 ± 1.1 nmol/L to a maximum of 5.8 ± 1.0 nmol/L after 4 hours of reduced maternal uterine blood flow (Fig. 1). The infusion of indomethacin caused a significant reduction in fetal plasma PGE_2 concentrations from a mean control value of 5.7 ± 1.7 nmol/L to below the sensitivity of the assay (in all except one experiment) 2 hours after starting the infusion. The addition of PGE_2 to the indomethacin infusion maintained fetal plasma PGE_2 concentrations at preinfusion levels; PGE_2 concentrations were similar before the start of the infusion (4.2 ± 1.4 nmol/L) compared with 6 hours after the start of the infusion (5.1 ± 1.6 nmol/L). Two hours after the completion of the indomethacin plus PGE_2 infusion fetal plasma PGE_2 concentrations had significantly decreased to 1.2 ± 1.0 nmol/L.

Blood gas and pH measurements. No effect of infusion type was found on the changes in fetal Sao_2, Pao_2 and $Paco_2$ in response to reduced maternal uterine blood flow, indicating that the level of hypoxemia was similar in all three groups (Fig. 2). During the vehicle infusion reduced maternal uterine blood flow significantly decreased fetal Pao_2 and Sao_2 from control values of 23.5 ± 0.2 mm Hg and 57.3% ± 3.0% to 14.7 ± 1.1 mm Hg and 23.9% ± 1.8%, respectively, after 1 hour. Similar decreases were observed during the other two infusions. In contrast, a significant effect of infusion type was found on the changes in pH_A in response to reduced maternal uterine blood flow. Before reduced maternal uterine blood flow was induced the indomethacin and indomethacin plus PGE_2 infusions, but not vehicle infusions, caused a significant reduction in pH_A compared with respective preinfusion control values (Fig. 2). During vehicle infusions reduced maternal uterine blood flow caused a significant reduction in pH_A from a control value of 7.36 ± 0.01 to a minimum of 7.20 ± 0.03 after 2 hours. Compared with vehicle infusions indomethacin infusions resulted in a lower pH_A at 1, 2, and 4 hours after inducing reduced maternal uterine blood flow (Fig. 2); fetal pH_A decreased from a mean control value of 7.36 ± 0.01 to a minimum of 7.10 ± 0.02 after 2 hours of reduced maternal uterine blood flow. The addition of PGE_2 to the indomethacin infusion reversed the effects of indomethacin alone during reduced maternal uterine blood flow (Fig. 2). During the indomethacin plus PGE_2 infusions, fetal pH_A decreased from a control value of 7.36 ± 0.01 to a minimum of 7.18 ± 0.02 at

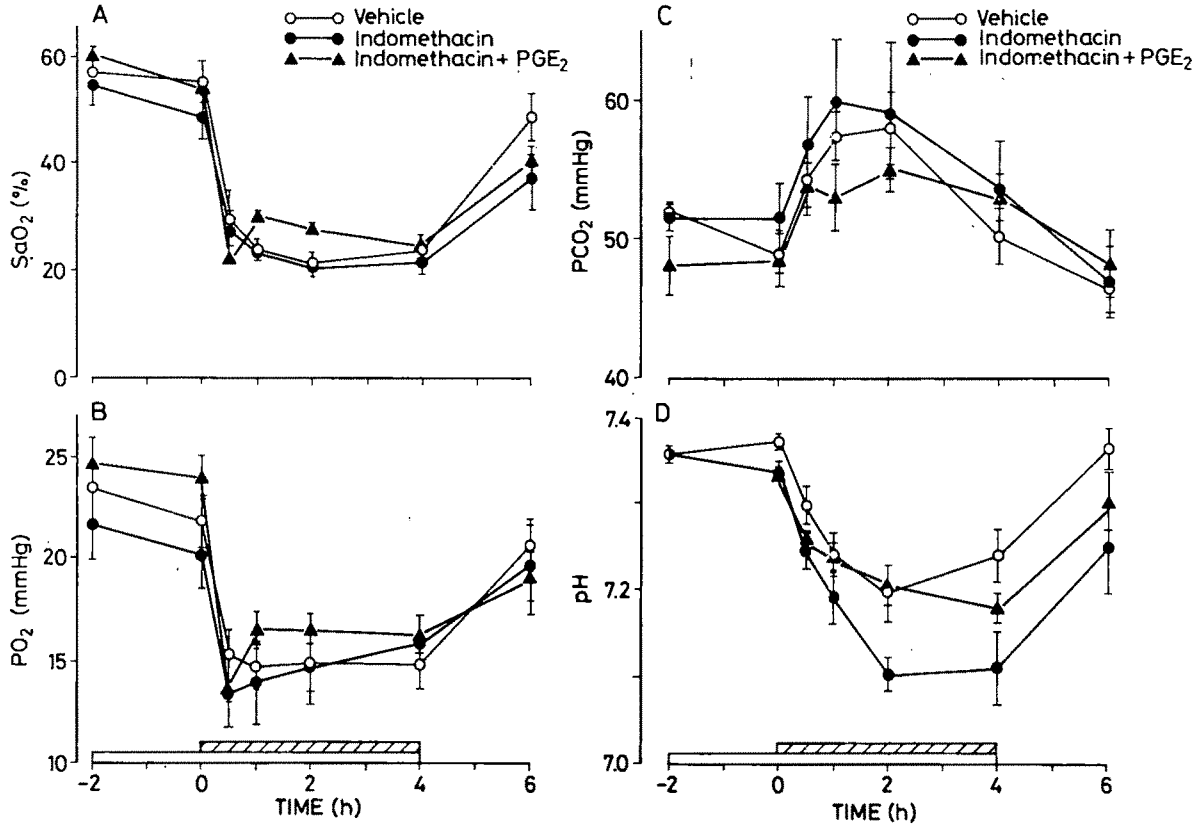


Fig. 2. SaO_2 **(A),** PaO_2 **(B),** $PaCO_2$ **(C),** and pH_A **(D)** measured during either a 6-hour vehicle *(open circles)*, indomethacin *(solid circles)*, or indomethacin plus PGE_2 *(triangles)* infusion. The period of reduced maternal uterine blood flow *(hatched bar)* coincided with the last 4 hours of the 6-hour infusions *(open bar)*.

4 hours after inducing reduced maternal uterine blood flow.

Fetal plasma glucose concentrations. A significant effect of infusion type was found on the changes in fetal plasma glucose concentrations. During vehicle infusions reduced maternal uterine blood flow caused a significant elevation in fetal plasma glucose concentrations from a preinfusion control value of 0.60 ± 0.09 mmol/L to a maximum of 1.1 ± 0.1 mmol/L after 30 minutes of reduced maternal uterine blood flow. Fetal plasma glucose concentrations remained elevated for the duration of the experiment (Fig. 3, *A*). Before reduced maternal uterine blood flow was induced, both the indomethacin and indomethacin plus PGE_2 infusions, but not vehicle infusion, caused a significant increase in fetal plasma glucose concentrations. During indomethacin infusions, glucose concentrations increased from a mean control value of 0.6 ± 0.04 to 1.3 ± 0.2 mmol/L (Fig. 3, *A*). Inducing reduced maternal uterine blood flow during the indomethacin infusion caused a further significant increase in fetal plasma glucose concentrations to a maximum of 1.8 ± 0.2 mmol/L after 1 hour of reduced maternal uterine blood flow. The addition of PGE_2 to the indomethacin infusion prevented the additional increase in glucose concentrations that occurred during reduced maternal uterine blood flow (Fig. 3, *A*).

Fetal plasma lactate concentrations. The three types of infusion had significantly different effects on fetal plasma lactate concentrations (Fig. 3, *B*). During vehicle infusions, fetal plasma lactate concentrations were significantly elevated by reduced maternal uterine blood flow from a control value of 2.4 ± 0.2 mmol/L to a maximum of 15.7 ± 1.7 mmol/L after 4 hours. The infusion of indomethacin before the induction of reduced maternal uterine blood flow caused a significant elevation in fetal plasma lactate concentrations from a preinfusion control value of 2.2 ± 0.1 to 6.7 ± 0.7 mmol/L. The induction of reduced maternal uterine blood flow during the indomethacin infusion caused a further significant elevation in fetal plasma lactate concentrations to a maximum of 22.7 ± 0.8 mmol/L after 4 hours of reduced maternal uterine blood flow. The addition of PGE_2 to the indomethacin infusion prevented the indomethacin-induced increase in fetal plasma lactate concentrations during normoxia and reduced the increase in lactate concentrations observed during an indomethacin infusion in the presence of reduced maternal uterine blood flow. The increases in fetal plasma lactate concentrations during reduced ma-

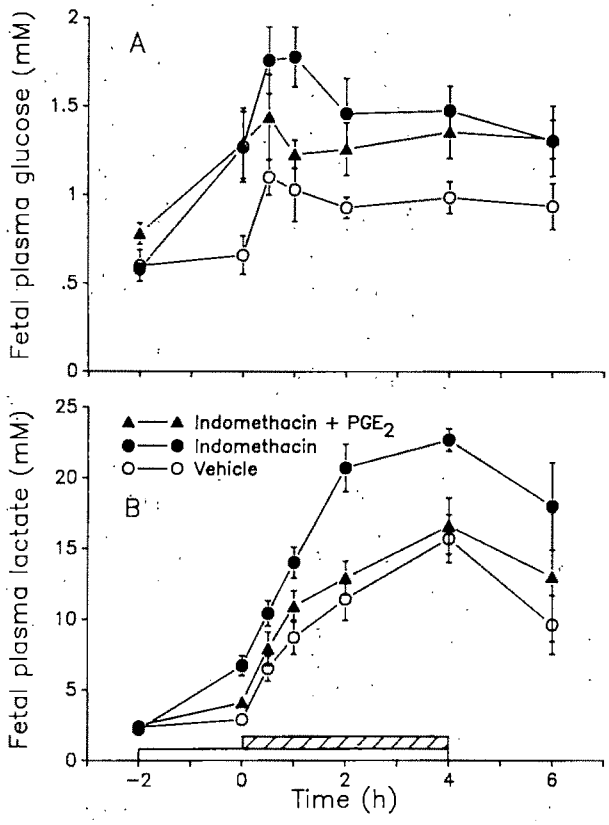


Fig. 3. Fetal plasma glucose **(A)** and lactate **(B)** concentrations measured during either a 6-hour vehicle *(open circles)*, indomethacin *(solid circles)*, or indomethacin plus PGE_2 *(triangles)* infusion. The period of reduced maternal uterine blood flow *(hatched bar)* coincided with the last 4 hours of the 6-hour infusions *(open bar)*.

ternal uterine blood flow were similar during vehicle infusions and indomethacin plus PGE_2 infusions.

Fetal plasma insulin concentrations. The three types of infusion had significantly different effects on the concentration of insulin in fetal plasma. The infusion of indomethacin alone had no significant effect on fetal plasma insulin concentrations during normoxia. During vehicle infusions, reduced maternal uterine blood flow caused a small reduction in fetal plasma insulin concentrations from a control value of 9.5 ± 0.7 μU/ml to a minimum of 5.9 ± 0.3 μU/ml after 4 hours of reduced maternal uterine blood flow (Fig. 4). Similarly, inducing reduced maternal uterine blood flow during indomethacin infusions caused a decrease in fetal plasma insulin concentrations from a control value of 14.7 ± 2.2 to a minimum of 6.5 ± 0.3 μU/ml after 2 hours of reduced maternal uterine blood flow. The addition of PGE_2 to the indomethacin infusion caused a large increase in fetal plasma insulin concentrations during normoxia from a preinfusion control value of 12.6 ± 0.7 to 60.9 ± 28.1 μU/ml after 2 hours. In the subsequent reduced maternal uterine blood flow period fetal plasma insulin concentrations decreased to a minimum value of 19.6 ± 5.2 μU/ml after 4 hours (Fig. 4).

Comment

In this study we have obtained evidence to support the contention that increased placental PGE_2 production plays an important role in the metabolic adaptation of the fetus to prolonged hypoxia. We have demonstrated that the inhibition of prostaglandin synthesis enhances the increase in fetal plasma glucose and lactate concentrations that occur during reduced maternal uterine blood flow. The enhanced increase in plasma lactate concentrations undoubtedly contributes to the severe reduction in fetal pH_A which, if sustained, causes fetal death (Hooper SB, Harding R, Thorburn GD. Unpublished observations). The addition of PGE_2 to the indomethacin infusion, however, prevented this augmented fetal acidemia, hyperglycemia, and hyperlactemia during reduced maternal uterine blood flow and stimulated a large increase in fetal plasma insulin concentrations.

During vehicle infusions PGE_2 concentrations were significantly elevated after 4 hours of reduced maternal uterine blood flow, which supports our earlier observation.[4] The infusion of indomethacin, a potent inhibitor of prostaglandin synthesis,[16] rapidly decreased fetal plasma PGE_2 concentrations; they remained low during the subsequent reduced maternal uterine blood flow period. The addition of PGE_2 to the indomethacin infusion maintained circulating fetal plasma PGE_2 concentrations at preinfusion levels for the duration of the infusion (Fig. 1). However, it is unlikely that the dose of PGE_2 administered was sufficient to sustain the normal delivery of PGE_2 to some tissues (e.g., liver) and, more importantly, to increase the delivery to a level that would occur during reduced maternal uterine blood flow.[4] The major source of circulating PGE_2 in the fetus is the placenta,[6, 7] and umbilical vein PGE_2 concentrations are much higher than the concentrations circulating in the fetus.[7] Because 75% of the blood supply to the fetal liver is derived from the umbilical vein,[17] the fetal liver is therefore normally exposed to higher concentrations of PGE_2 than the concentrations that circulate in fetal blood. This could explain why PGE_2 did not reverse all of the effects of insulin administration.

The infusion of indomethacin during reduced maternal uterine blood flow caused a much greater reduction in pH_A than when the vehicle was infused. During reduced maternal uterine blood flow the reduction in pH_A most probably results from the elevation in $Paco_2$ (respiratory acidosis), the elevation in blood lactate, and possibly fatty acid concentrations (metabolic acidosis), although these were not measured. The greater reduction in pH_A during reduced maternal

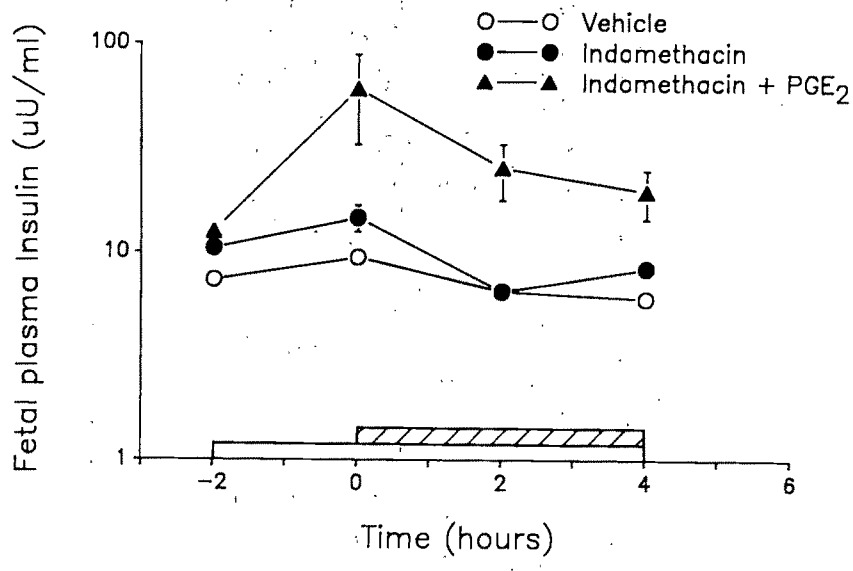


Fig. 4. Fetal plasma insulin concentrations plotted on a log scale, measured during either a 6-hour vehicle *(open circles)*, indomethacin *(solid circles)*, or indomethacin plus PGE_2 *(triangles)* infusion. The period of reduced maternal uterine blood flow *(hatched bar)* coincided with the last 4 hours of the 6-hour infusions *(open bar)*.

uterine blood flow in indomethacin compared with vehicle infusions must have been metabolic in origin because the elevation in $PaCO_2$ was similar in both experiments, whereas blood lactate concentrations were greater with indomethacin. The addition of PGE_2 to the indomethacin infusion prevented this enhanced acidosis, presumably by preventing the increase in lactate concentrations; however, it is also possible that PGE_2 reduced fatty acid concentrations by decreasing lipolysis in adipose tissue.[18] If reduced maternal uterine blood flow and the indomethacin infusions were continued for a longer period of time (>6 hours), these fetuses would probably have died. In two preliminary experiments we considered it appropriate to induce reduced maternal uterine blood flow during an indomethacin infusion for at least 12 hours because PGE_2 increases relatively slowly during reduced maternal uterine blood flow and is significant only after 4 hours.[4] In these preliminary experiments both fetuses became severely acidotic (pH 6.9 to 7.0) and died between 6 and 8 hours after inducing reduced maternal uterine blood flow (Hooper, Harding, and Thorburn, unpublished observations). This observation and those presented in our study clearly demonstrate the detrimental effects that indomethacin has on a hypoxemic fetus. Furthermore, these effects could explain the unaccounted fetal deaths that have occurred in utero after the administration of indomethacin to prevent premature labor in human beings.[19]

The control of blood glucose concentrations in the fetus is primarily dependent upon maternal blood glucose concentrations, insulin production by the fetal pancreas, fetal glucose use, and glucose production or storage by the liver.[20] Gluconeogenesis is a very important source of blood glucose in adult ruminants,[21] but although the fetal sheep liver has all the necessary enzymes it is believed that this metabolic pathway does not operate until after birth.[22, 23] It is not known, however, whether this pathway can be activated during fetal hypoxemia. The finding in this study that an indomethacin infusion during normoxia causes an elevation in fetal plasma glucose concentrations has been reported previously.[24] The mechanism underlying this increase is not known although it probably results from an increase in glucose release from the liver because insulin concentrations were not altered by indomethacin.

PGE_2 failed to reverse the effect of indomethacin on fetal blood glucose concentrations during normoxia, which could mean that (1) the dose of PGE_2 administered was insufficient to increase PGE_2 concentrations in the umbilical vein to normal levels, (2) PGE_2 is the wrong prostaglandin, or (3) the failure was due to a side effect of indomethacin unrelated to prostaglandin synthesis. One known side effect of indomethacin is the inhibition of phosphodiesterase activity, although this only occurs at much higher doses than that used in our study.[25] Inhibition of phosphodiesterase activity would cause a sustained elevation in intracellular cyclic adenosine monophosphate (cAMP) concentrations by preventing the breakdown of cAMP. As discussed below, an increase in intracellular cAMP concentrations in liver tissue would decrease glycolysis and promote gluconeogenesis and glucose release.[26] Other known

side effects of indomethacin are oligohydramnios,[5, 27] possibly caused by reduced fetal urine production, a reduction in cerebral blood flow in fetal sheep,[24] and an increase in vascular tone of the ductus arteriosus.[28]

Fetal plasma catecholamine concentrations are elevated by fetal hypoxia whether it is induced by maternal hypoxia[29] or reduced maternal uterine blood flow.[4] The increase in fetal plasma catecholamine concentration is believed to be responsible for the increase in glucose concentration during fetal hypoxemia.[30] Catecholamines promote an increase in glucose release by the liver, which is mediated by β-adrenergic receptors and an increase in cAMP levels.[31] Catecholamines also inhibit insulin release from the pancreas, an action mediated by α-adrenergic receptors and a decrease in cAMP levels.[18] The increases in fetal plasma glucose concentrations we observed during reduced maternal uterine blood flow (vehicle infusion) are similar to those reported previously during fetal hypoxia caused by maternal hypoxia.[30] The infusion of indomethacin, however, caused a greater increase in fetal blood glucose concentrations after the induction of reduced maternal uterine blood flow. The mechanism by which indomethacin enhances this increase in fetal blood glucose during reduced maternal uterine blood flow is not known. It is highly likely, however, to be mediated by an inhibition of PGE_2 synthesis because the addition of PGE_2 to the indomethacin infusion prevented this increase. We suggest that PGE_2 may antagonize some of the biochemical actions of the catecholamines in utero by exerting opposing actions on tissue levels of cyclic adenosine monophosphate. For example, we speculate that PGE_2 acts on the fetal liver to lower tissue cAMP concentrations. Increased cAMP levels in the liver decrease glycolysis and promote an increase in gluconeogenesis and glycogenolysis, leading to increased hepatic glucose release. If PGE_2 acts on the fetal liver as it does in adipose tissue,[18] increased PGE_2 concentrations would promote a reduction in liver cAMP levels; this would enhance glycogen synthesis and glycolysis and inhibit glycogenolysis and gluconeogenesis, thereby decreasing glucose release. Thus reducing circulating PGE_2 concentrations during reduced maternal uterine blood flow would lead to an unopposed action of the catecholamines on liver cAMP levels, leading to increased glucose production.

The concept that PGE_2 can oppose the action of catecholamines in utero is supported by the finding that PGE_2 release from the placenta prevents brown-fat metabolism (nonshivering thermogenesis) in the fetus.[11] In newborn lambs brown-fat metabolism is activated by adrenergic stimulation and is mediated by β-adrenergic receptors, which increase cAMP concentrations.[32] PGE_2 is known to inhibit lipolysis in adipose tissue by reducing cAMP concentrations[18] and probably has a similar action on brown adipose tissue to prevent nonshivering thermogenesis in the fetus.[11] In our study, in which fetal PGE_2 concentrations were reduced by indomethacin and sympathoadrenal activity was increased by reduced maternal uterine blood flow,[4] it seems likely that lipolysis would have been stimulated and that the release of fatty acids would have contributed to the observed acidosis.

The action of catecholamines on the β-islet cells of the pancreas to inhibit insulin release is mediated by α-adrenergic receptors leading to a reduction in cAMP concentrations.[26] In these experiments PGE_2 stimulated a large increase in insulin concentrations, again demonstrating an opposing action to the catecholamines. We speculate that PGE_2 acts on membrane-bound adenylcyclase receptors, resulting in an increase in cAMP concentrations and thus insulin release.

The indomethacin infusion during normoxia caused an increase in fetal plasma lactate concentrations, which probably resulted from the increase in plasma glucose concentrations. The addition of PGE_2 to the indomethacin infusion prevented this increase in lactate concentrations, probably because of the large increase in plasma insulin concentrations and possibly also because of the suppression of glycogenolysis. Increased plasma insulin concentrations would promote the uptake of glucose for oxidative metabolism or storage as glycogen. During fetal hypoxia the increase in fetal plasma glucose concentrations is believed to be largely responsible for the increase in fetal blood lactate concentrations because of the anaerobic metabolism of fetal glucose primarily by the placenta.[30] Thus the increased fetal blood glucose could account for the enhanced increase in circulating fetal plasma lactate concentrations caused by indomethacin during reduced maternal uterine blood flow. The addition of PGE_2 prevented this effect of indomethacin during reduced maternal uterine blood flow, probably by elevating insulin concentrations and suppressing glucose production.

We have demonstrated that during fetal hypoxemia indomethacin has profound metabolic effects on the fetus; if the hypoxia or infusion is prolonged, the fetus is at risk of fatal acidemia. A similar finding has been observed after the inhibition of prostaglandin synthesis during another form of fetal stress, hyperthermia.[9] During hyperthermia the infusion of 4-aminoantipyrine, a prostaglandin synthetase inhibitor, caused a large increase in fetal plasma glucose concentrations and a rapid decrease in fetal pH; 4 of 10 fetuses died from this treatment.[9] We contend, therefore, that an elevation in placental prostaglandin synthesis, probably PGE_2, plays an integral role in the fetal metabolic adaptation to stress, possibly by leading to a gradual suppression of the metabolic actions of the catecholamines.

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Tumor necrosis factor in preterm and term labor

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OBJECTIVE: Our objective was to determine if labor (term and preterm) and microbial invasion of the amniotic cavity were associated with changes in amniotic fluid concentrations of tumor necrosis factor.
STUDY DESIGN: Amniotic fluid was retrieved by transabdominal amniocentesis from 269 women in the following groups: midtrimester (n = 38), preterm labor with intact membranes (n = 52), preterm premature rupture of membranes (n = 74), term in active labor (n = 34), and term not in labor (n = 21). Fluid was cultured for aerobic and anaerobic bacteria and for *Mycoplasma* species. Tumor necrosis factor was measured with a commercially available enzyme-linked immunosorbent assay validated for amniotic fluid (sensitivity 60 pg/ml).
RESULTS: Amniotic fluid from pregnant women in the second and third trimesters who were not in labor did not contain tumor necrosis factor. Among women in preterm labor, 92.3% (12/13) of patients with a positive amniotic fluid culture had detectable tumor necrosis factor in the amniotic fluid (median 820 pg/ml, range <60 to 2340 pg/ml). In contrast, only 10.2% (4/39) of women with a negative amniotic fluid culture had detectable tumor necrosis factor. Histopathologic chorioamnionitis was found in all patients who had a positive amniotic fluid culture, and tumor necrosis factor was detectable in the amniotic fluid of all but one of these patients. Among women in active labor at term, 25% (21/84) had detectable tumor necrosis factor in the amniotic fluid. Tumor necrosis factor was detected more frequently in the amniotic fluid of patients with a positive amniotic fluid culture than in patients with a negative culture (46.6% [7/15] vs 20.2% [14/69], p = 0.047). Amniotic fluid concentrations of tumor necrosis factor were significantly higher in patients with preterm premature rupture of membranes, labor, and a positive amniotic fluid culture than in the other subgroups of patients with preterm premature rupture of membranes.
CONCLUSION: Parturition in the setting of microbial invasion of the amniotic cavity is associated with activation of the cytokine network as demonstrated by the detection of tumor necrosis factor in human amniotic fluid. (AM J OBSTET GYNECOL 1992;166:1576-87.)

Key words: Labor, parturition, amniotic fluid, tumor necrosis factor, prematurity, preterm labor, term labor, immunoassay, cytokine

There is a strong association between systemic and intrauterine infection and preterm labor and delivery.[1] A growing body of evidence suggests that cytokines, which are capable of stimulating prostaglandin production by intrauterine tissues, may serve as mediators of parturition in the setting of infection.[2-5] Tumor necrosis factor (TNF) is a cytokine produced by activated macrophages in response to a wide variety of stimuli, including bacterial products, viruses, and parasites. It has been proposed that this cytokine may participate in the mechanisms of human parturition associated with infection.[4] TNF is produced by human decidua in response to bacterial products[6, 7] and stimulates prostaglandin production by amnion[4] and decidua.[5]

Previously, we have identified the presence of TNF in the amniotic fluid of women with microbial invasion of the amniotic cavity and preterm labor by means of the L929 bioassay.[4] This bioassay, like others for cytokines, is time consuming, laborious, semiquantitative, and is affected by the presence of inhibitors in biologic fluids. Clinical application of TNF determinations to obstetric practice requires the availability of sensitive, rapid, and simple assays for its detection. Recently, immunoassays for this cytokine have become available. The purpose of this study was to validate an immunoassay for the quantitation of TNF in human amniotic fluid and to examine the changes in amniotic fluid concentrations of this cytokine in the setting of labor (term and preterm) and microbial invasion of the amniotic cavity.

Material and methods

Study design. A cross-sectional study was conducted according to the results of amniotic fluid cultures, ges-

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tational age, and labor status. Five groups were identified for study purposes (Table I). Group 1 consisted of women in the midtrimester of pregnancy (gestational age 16 to 18 weeks) who were undergoing amniocentesis for genetic indications (maternal age >35 years) (n = 38), and group 2 consisted of women at term (38 to 39 weeks) who had amniocentesis for the assessment of fetal lung maturity before elective cesarean section (n = 21). All these patients had a negative amniotic fluid culture. Group 3 consisted of women admitted with preterm labor and intact membranes (n = 52); this group was subdivided according to the results of amniotic fluid culture and response to tocolysis: women with a negative amniotic fluid culture who responded to tocolysis and were delivered at term (n = 23), women with a negative culture who failed to respond to tocolysis and were delivered of a preterm neonate (n = 16), and women with a positive culture who were delivered prematurely (n = 13). Group 4 consisted of women with preterm premature rupture of membranes (n = 74). This group was subdivided according to the presence of labor at the time of amniocentesis and the results of the amniotic fluid culture: women with a negative culture and without preterm labor (n = 26); women with a negative culture but with preterm labor (n = 15); women with a positive culture but without preterm labor (n = 11), and women with a positive culture and preterm labor (n = 22). Group 5 consisted of women in spontaneous active labor at term with intact membranes (n = 84). This group was subdivided according to the results of amniotic fluid cultures: patients with a negative culture (n = 69) and patients with a positive culture (n = 15).

Premature rupture of membranes was defined as amniorrhexis before the onset of spontaneous labor. Nitrazine, pooling, and ferning tests were performed in all women in labor to exclude leakage of amniotic fluid into the vagina. Premature rupture of membranes was diagnosed if at least two of the three tests were positive. Pelvic examinations were not performed at the time of amniocentesis, because this could result in an increased risk of infection.[8] Tocolytic agents were not used in patients with preterm premature rupture of membranes.

Preterm labor was defined as the presence of regular uterine contractions with a frequency of at least two every 10 minutes and with changes in the cervical Bishop's score occurring before 37 completed weeks. Ritodrine was administered intravenously as the tocolytic agent to all patients according to a protocol described elsewhere.[9] Amniocentesis was performed before the initiation of therapy. Failure of tocolysis was diagnosed when cervical dilatation progressed beyond 5 cm or delivery occurred.

Retrieval of amniotic fluid. Amniotic fluid was retrieved by transabdominal amniocentesis in all women in this study. Amniocentesis is offered in our institution to all patients with the diagnosis of preterm labor for microbiologic and fetal lung maturity studies. Women in active labor at term (group 5) were ascertained by searching our obstetric data base. They were identified as patients who met the following criteria: (1) amniocentesis performed because of suspected preterm labor with intact membranes on the basis of uncertain dates, (2) results of amniotic fluid studies (lecithin/sphingomyelin ratio, foam stability test, shake test) consistent with fetal lung maturity, (3) delivery within 24 hours of amniocentesis, (4) neonatal birth weight >2500 gm, (5) pediatric examination (Dubowitz method) consistent with a gestational age >37 weeks, and (6) absence of respiratory distress syndrome or any other neonatal complication attributable to preterm birth. The identification number of patients fulfilling this criterion was cross-referenced with samples stored in our amniotic fluid bank.

Table I. Patient population

Group	*No.*
Group 1: Midtrimester	38
Group 2: Term, no labor	21
Group 3: Preterm labor, intact membranes	52
No infection, no delivery	23
No infection, delivery	16
Infection, delivery	13
Group 4: Preterm premature rupture of membranes	74
No infection, no labor	26
No infection, labor	15
Infection, no labor	11
Infection, labor	22
Group 5: Term, active labor	84
No infection	69
Infection	15

A sample of amniotic fluid was sent for microbiologic studies. The remaining fluid was centrifuged at 200g for 10 minutes at 4° C, divided in units of 300 μl per vial, and stored in polypropylene tubes at −70° C until assayed. Samples were not subjected to freeze-thaw cycles before assay.

Microbiologic culture technique. Amniotic fluid was transported to the laboratory in a capped plastic syringe immediately after collection. These conditions precluded air contact with the specimen. Plating occurred within 30 minutes of collection in all cases. Amniotic fluid was cultured for aerobic and anaerobic bacteria and *Mycoplasma* by methods previously described.[10] The Gram stain of amniotic fluid was used for patient management. Patients with a positive Gram stain received antibiotics (generally ampicillin and gentamicin) and had tocolysis discontinued.

Quantitation of TNF. Amniotic fluid TNF was measured with a commercially available, enzyme-linked immunoassay (ELISA) (FactorTest-Human TNF-α, Gen-

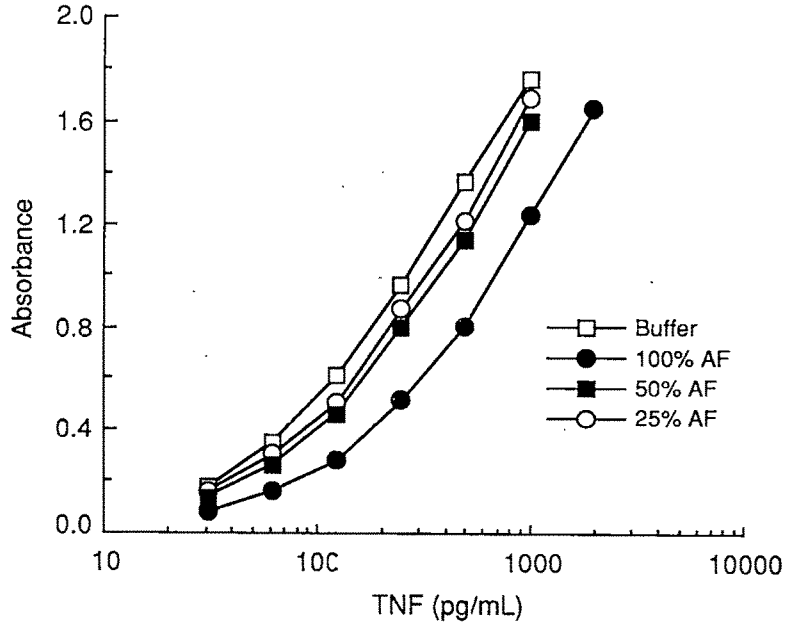


Fig. 1. Results of spike and recovery experiments. Serial twofold dilutions of recombinant human TNF-α were added to assay buffer and pooled amniotic fluid. Final concentrations of pooled fluid were 25%, 50%, and 100%. Best recovery of spiked human recombinant TNF-α was obtained when final concentration of amniotic fluid was 25%. *AF,* Amniotic fluid.

Table II. Parallelism studies in amniotic fluid*

Sample dilution	*Observed TNF values (pg/ml)*	*Expected TNF values (pg/ml)*	*Recovery† (%)*
Experiment 1			
1:4	240	240	100
1:8	110	120	92
1:16	48	60	80
Experiment 2			
1:4	240	240	100
1:8	130	120	108
1:16	53	60	88

*Assay was performed as outlined in Material and Methods. Values displayed are the average of duplicate determinations.
†Recovery (%) = Observed value/Expected value × 100.

zyme Corporation, Cambridge, Mass.). The assay is a triple-antibody sandwich using a monoclonal anti-TNF-α antibody as the capture reagent, rabbit polyclonal anti-TNF-α, biotinylated goat antirabbit immunoglobulin with streptavidin-peroxidase, and o-phenylenediamine as the chromogen. The linear range of the assay standard curve is 25 to 400 pg/ml of TNF-α in assay wash buffer, with a detection limit of 12 pg/ml of TNF-α in assay wash buffer. The assay system has no detectable cross reactivity with ≥2 ng/ml of interleukin-1α, interleukin-1β, interleukin-2, interleukin-3, interleukin-4, interleukin-6, granulocyte-macrophage colony-stimulating factor, macrophage colony-stimulating factor, gamma interferon, and TNF-β (lymphotoxin).

ELISA validation for quantitation of TNF-α in amniotic fluid. Pooled amniotic fluid samples from normal individuals (negative bacterial culture) that did not contain detectable TNF in the L929 bioassay were used for the following validation studies:

1. Spike and recovery. Graded amounts of human recombinant TNF-α were added to 25%, 50%, and 100% amniotic fluid diluted in assay wash buffer (phosphate-buffered saline solution, Tween 20, and 0.5% protein). From these data it was determined that amniotic fluid should be diluted 1:4 to eliminate matrix effects (Fig. 1).
2. Parallelism. An amniotic fluid sample that was positive for TNF by previous ELISA assay was diluted 1:4 with wash buffer, then further diluted in wash buffer. Parallelism was determined by calculating the observed versus expected TNF concentrations (Table II).
3. The minimal detectable concentration of TNF-α was determined to be 60 pg/ml in amniotic fluid when diluted 25% with wash buffer. This value was defined as the amount of TNF-α that provided absorbance values that were 2 SDs above the mean absorbance of 10 replicate determinations of the diluent alone.
4. The coefficient of variation for intraassay precision was determined to be ≤6% by replicate determinations of six different specimens (serial dilutions from 62 to 1000 pg/ml of TNF-α diluted

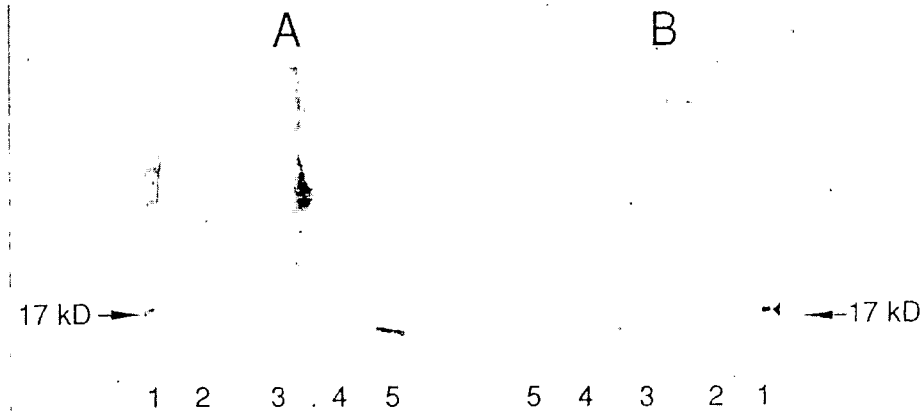


Fig. 2. Three amniotic fluid samples were assayed by immunoblot. Lanes contain: *1*, molecular weight marker; *2*, amniotic fluid containing 2000 pg/ml TNF-α (determined by ELISA) from a patient with intraamniotic infection; *3*, normal midtrimester amniotic fluid (no detectable TNF-α by ELISA); *4*, normal term amniotic fluid (no detectable TNF-α by ELISA); *5*, recombinant TNF-α. Immunoreactive bands are visible at 17 kd, which is characteristic of TNF-α, in *lanes 1* and *5* in blot *A*. Blot *A* was reacted with rabbit anti-TNF-α antiserum. In contrast, these bands are not detectable in blot *B*, which was reacted in parallel with normal rabbit serum.

in 25% amniotic fluid). Ten replicates of each specimen were tested in a single assay.

5. The coefficient of variation for interassay precision was determined to be ≤14% by replicate determinations of six different samples ranging in TNF concentration from 25 to 350 pg/ml in three individually calibrated assays.
6. The specific detection of TNF in the ELISA was confirmed by substitution of an irrelevant matched isotype (immunoglobulin G1) monoclonal (DMS-1, which binds human interleukin-2 [Genzyme Corporation]) in place of the capture reagent included in the kit. Amniotic fluid with TNF signals in the specific ELISA up to 2000 pg/ml had no detectable TNF when tested in the assay that included the irrelevant monoclonal.

Immunoblot and bioassay confirmation of TNF-α presence in amniotic fluid.

Immunoblot. Selected samples of amniotic fluid with ≥2000 pg/ml TNF-α (as determined by ELISA) were analyzed by immunoblot (WestTest Human TNF-α, Genzyme Corporation). Briefly, amniotic fluid samples were diluted 1:1 with an sodium dodecyl sulfate-2-mercaptoethanol–containing buffer and applied in duplicate wells to a 15% sodium dodecyl sulfate–polyacryamide gel. After electrophoresis, the samples were transferred to nitrocellulose in Tris-glycine buffer, pH 8.5, containing 20% methanol. The finished blot was cut in half vertically. Each half was blotted, then incubated with either rabbit polyclonal antihuman TNF-α or with preimmune rabbit serum. Both halves were then developed with protein G-biotin, streptavidin-peroxidase, and aminoethylcarbonyl chromogen. A 17 kd immunoreactive protein (Fig. 2, panel A) was detected when assayed with the relevant rabbit anti-TNF antibody. In contrast, this 17 kd protein was not detected when parallel samples were incubated with preimmune rabbit serum instead of the relevant primary anti-TNF antisera (Fig. 2, panel B).

L929 cell bioassay. The cytostatic activity of TNF-α was measured on mouse L929 fibroblasts[11] with the vital stain 3-[4,5-dimethylthiazol-2-yl]-3,5-diphenyltetrazolium bromide (MTT) (Sigma Chemical Co., St. Louis). Briefly 5×10^4 L929 cells obtained from log phase culture were added to each well of a 96-well microtiter plate, and cultured overnight at 37° C in a 5% carbon dioxide incubator. Subsequently, culture medium was removed and replaced with graded concentrations of recombinant human TNF-α (Genzyme H-TNF) (specific activity 10^7 U/mg) in complete medium, patient samples (after 1:4 dilution in complete medium and passage through a 0.2 μm sterile filter), or complete medium as a negative control. All additions contained 1 μg/ml actinomycin D (Sigma). Cultures were then incubated at 37° C in 5% carbon dioxide for 16 hours.

After the second incubation, 100 μl of culture medium was removed from each well, and 20 μl of MTT (5 mg/ml in sterile phosphate-buffered saline solution) (Sigma) was added. Cultures were incubated for an additional 4 hours at 37° C in 5% carbon dioxide, and then 120 μl of 0.4N hydrochloric acid in 2-propanol was added to each well. Cells were resuspended to dissolve MTT crystals and resultant absorbance was read at 595 nm. Bioactivity expressed by patient samples was quantified by comparison with the standard curve generated with recombinant human TNF-α.

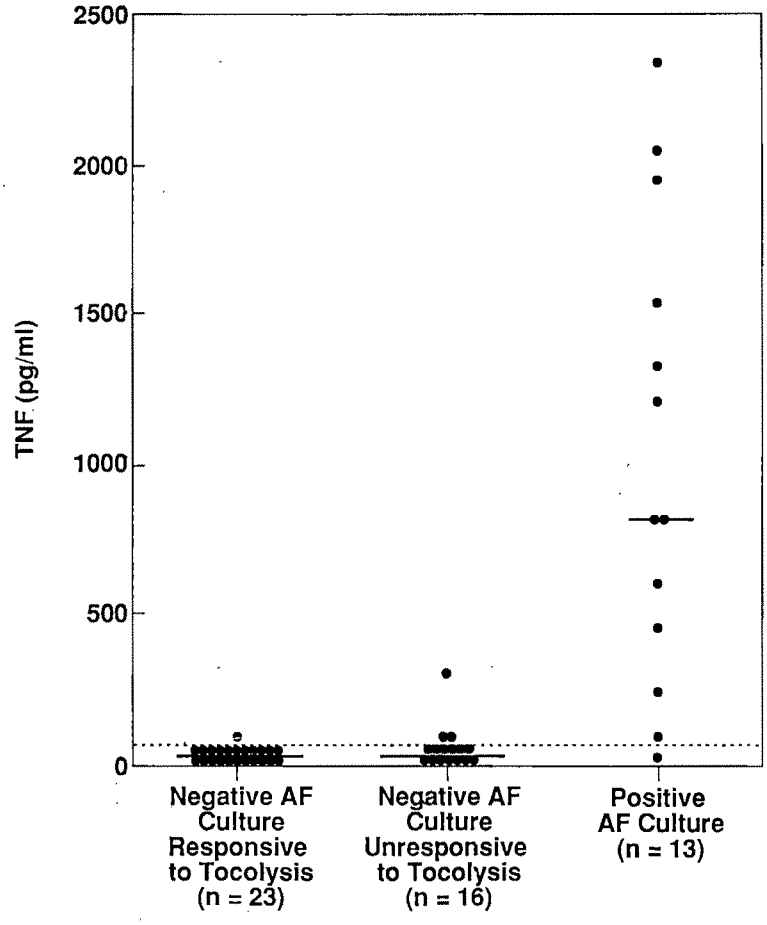


Fig. 3. Amniotic fluid TNF in women with preterm labor and intact membranes. TNF was detected more frequently in women with positive amniotic fluid culture than in women with negative culture in the other two groups ($p < 0.01$, Fisher's exact test). *AF*, Amniotic fluid.

Neutralization of TNF-α bioactivity. Specificity of cytotoxicity was confirmed with neutralizing anti-TNF antibody (IP-300, Genzyme Corporation). Selected patient samples were preincubated at 37° C with a 1:100 dilution of rabbit antihuman TNF-α antisera, whereas parallel control samples received a 1:100 dilution of normal rabbit serum. Antibody-sample mixtures were then applied to an L929 bioassay, and TNF bioactivity was determined as described.

L929 cell bioassay indicated the presence of 925 pg/ml of TNF-α bioactivity (as determined from the bioassay standard curve) in a sample that contained 865 pg/ml of TNF-α as determined in ELISA. The bioactivity in this sample was totally neutralized by pretreatment with a 1:100 dilution of rabbit anti-TNF-α neutralizing antibody (code IP-300, Genzyme Corporation). Also, both amniotic fluid samples for which TNF was undetectable in the ELISA failed to show the 17 kd band on Western blot and contained insignificant levels of cytotoxicity in the L929 bioassay.

Statistical analysis. Comparisons between proportions were performed with either a χ^2 or Fisher's exact test. An unpaired Student t test was used to compare continuous variables that were normally distributed (gestational age, maternal age). A Wilks-Shapiro test was used to test the hypothesis that amniotic fluid TNF concentrations were normally distributed. A Kruskal-Wallis analysis of variance was used to compare amniotic fluid TNF concentrations among study groups. Dunn's test was used for multiple post hoc comparisons (True Epistat, Epistat Services, Richardson, Tex.).

Results

Amniotic fluid from women in the midtrimester of pregnancy and women at term who were not in labor did not contain detectable TNF. Fig. 3 displays amniotic fluid concentrations of TNF in women in preterm labor with intact membranes. TNF was detected in 92.3% (12/13) of patients with a positive amniotic fluid culture for microorganisms (median 820 pg/ml, range <60 to 2340 pg/ml). Only three of 16 (18.7%) women in preterm labor who had negative amniotic fluid cultures for

Table III. Amniotic fluid microbiologic and placental pathologic studies and amniotic fluid TNF concentration in women with positive amniotic fluid cultures and preterm labor

Patient No.	*Gestational age (wk)*	*Gram stain*	*Culture*	*Quantitative microbiology (cfu/ml)*	*Histologic chorioamnionitis*	*TNF (pg/ml)*
1	25	−	*Ureaplasma urealyticum*	−	+	<60
2	32	−	*Streptococcus agalactiae*	−	NA	2040
3	30	+	*Gardnerella vaginalis*	$>10^5$	NA	2340
4	26	+	*Capnocytophaga sp.*	−	+	820
			Clostridium sp.	4+		
5	22	+	*Gardnerella vaginalis*	$>10^5$	+	600
6	24	−	*Streptococcus viridans*	−	+	100
7	25	+	*Candida sp.*	$>10^5$	+	1540
8	33	+	*Fusobacterium sp.*	−	+	240
9	28.5	+	*Fusobacterium sp.*	$>10^5$	NA	1320
			Mycoplasma hominis	−		
			Ureaplasma urealyticum	−		
10	28	+	*Gardnerella vaginalis*	$>10^5$	+	1950
11	28	−	*Ureaplasma urealyticum*	−	NA	460
12	26	−	*Fusobacterium sp.*	−	NA	1210
13	24	+	*Streptococcus agalactiae*	$>10^5$	+	820
			Ureaplasma urealyticum	−		

NA, Not available.

microorganisms and premature delivery had detectable TNF in amniotic fluid. Of these patients, one had a positive amniotic fluid Gram stain for microorganisms. The second patient had a negative Gram stain but an amniotic fluid white blood cell count of 3920/mm³ with 80% granulocytes and severe histologic chorioamnionitis. There was no statistical difference in the interval between amniocentesis and delivery in patients with positive and negative amniotic fluid cultures in this group (median 6.75 hours, range 0.7 to 20 hours vs median 10 hours, range 1.5 to 21 hours, respectively; $p > 0.05$, Mann-Whitney U test). In the remaining group (negative amniotic fluid culture and delivery at term), only one patient of 23 (4.3%) had detectable TNF (100 pg/ml).

Table III displays the results of microbiologic analysis, Gram stain of amniotic fluid, histopathologic examination of the placenta, and amniotic fluid TNF concentrations of the 13 patients with a positive amniotic fluid culture. Gram stain of amniotic fluid was positive in only 61.5% (8/13) of patients. The most common microbial isolates were *Ureaplasma urealyticum* ($n = 4$), *Fusobacterium* sp. ($n = 3$), and *Gardnerella vaginalis* ($n = 3$). Polymicrobial invasion was present in 23% (3/13) of patients. None of the mothers had clinical chorioamnionitis. One newborn delivered at 26 weeks (birth weight 810 gm) had bacterial meningitis (patient 4 in Table III). Histopathologic chorioamnionitis was detectable in all patients who had positive amniotic fluid cultures and who had placental examination ($n = 8$). All these patients but one had detectable TNF in amniotic fluid. Placental examination was performed in six of 16 patients who were delivered of a preterm neonate and had a negative amniotic fluid culture. Four of these placentas (66.6%) had histologic evidence of chorioamnionitis. Only one of these patients had detectable TNF in amniotic fluid.

Fig. 4 displays amniotic fluid concentrations of TNF in women with preterm premature rupture of membranes. Women with preterm labor and positive amniotic fluid cultures had significantly higher TNF concentrations (median 249 pg/ml, range <60 to 1980 pg/ml) than did women in the other groups ($p < 0.01$). TNF was detected overall in 45.4% (15/33) of patients with a positive culture and in 2.4% (1/41) of those with a negative culture.

Table IV displays the results of microbiologic analysis, Gram stain of amniotic fluid, and amniotic fluid TNF concentrations of the 22 patients with preterm premature rupture of membranes, labor, and positive amniotic fluid culture. Table V displays similar data from 11 women with preterm premature rupture of membranes and positive amniotic fluid cultures who were not in labor. No significant difference in the proportion of women with microbial invasion exclusively caused by *Mycoplasma*, with gram-negative microorganisms or with polymicrobial infections, could be detected between the two groups. Gram stain was positive in 33.3% (11/33) of patients. Clinical chorioamnionitis was present in three patients and histologic chorioamnionitis in 16 of 20 patients in whom placental examination was performed. There were no cases of neonatal sepsis in this group.

Fig. 5 displays amniotic fluid concentrations of TNF in women at term. Overall, 25% (21/84) of women in active labor had detectable amniotic fluid TNF. Amniotic fluid TNF was detected more frequently in patients with a positive culture than in those with a negative culture (46.6% [7/15] vs 20.2% [14/69], respectively; $p = 0.047$, Fisher's exact test). Among women

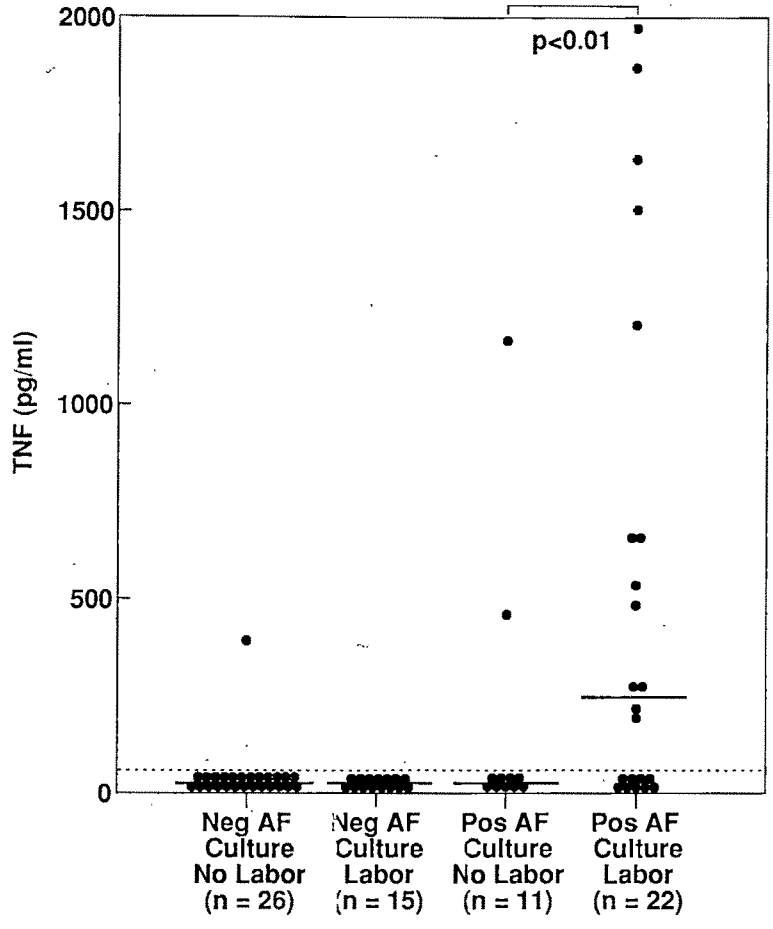


Fig. 4. Amniotic fluid TNF in women with preterm premature rupture of membranes. TNF was detected more frequently in women with positive amniotic fluid culture and preterm labor than in women in the other groups ($p < 0.001$). *AF*, Amniotic fluid.

in labor with detectable amniotic fluid TNF, those with a positive culture had a significantly higher median TNF than those with a negative culture (median 200 pg/ml, range 95 to 815 pg/ml vs median 105 pg/ml, range 60 to 660 pg/ml, respectively; $p = 0.03$, Mann-Whitney U test). Also, TNF was detected more frequently in women in labor than in women not in labor (25% [21/84] vs 0% [0/21], respectively; $p = 0.0063$, Fisher's exact test).

The most common microorganisms isolated at term were *U. urealyticum* ($n = 11$, 73.3%) followed by *Lactobacillus* sp. ($n = 2$, 13.3%). Polymicrobial invasion of the amniotic cavity was present in 20% of patients (3/15). There was no difference in cervical dilatation in patients with positive and negative amniotic fluid cultures (median 2 cm, range 0 to 6 cm vs median 2 cm, range 0 to 5 cm, respectively; $p > 0.05$, Mann-Whitney U test). Five patients had puerperal endometritis and only one of them had detectable amniotic fluid TNF. There were no cases of clinical chorioamnionitis or neonatal sepsis in this group. Placental pathologic studies were not available in these cases (probably because most newborns weighed >2800 gm and had no immediate neonatal complications).

Among women with microbial invasion of the amniotic cavity and labor, those in preterm labor had significantly higher amniotic fluid concentrations of TNF than those in term labor (median 820 pg/ml, range <60 to 2340 pg/ml vs median 47.5 pg/ml, range <60 to 815 pg/ml; $p < 0.0004$, Mann-Whitney U test) (Fig. 6).

Comment

Our data indicate that amniotic fluid from normal women does not contain detectable immunoreactive TNF. This finding is consistent with previous data generated with the use of bioassays for the detection of this cytokine.[4, 6] In contrast to other cytokines such as interleukin-6[12] and macrophage colony-stimulating factor,[13] TNF is not a normal constituent of amniotic fluid. Rather, TNF belongs to another class of cytokines (e.g., interleukin-1α and interleukin-1β), which are expressed in amniotic fluid under pathologic conditions (i.e., infection or inflammation)[2, 14] or

Table IV. Amniotic fluid microbiologic and placental pathologic studies and amniotic fluid TNF concentrations in women with premature rupture of membranes and preterm labor

Patient No.	*Gestational age (wk)*	*Gram stain*	*Culture*	*Quantitative microbiology*	*Histologic chorioamnionitis*	*TNF (pg/ml)*
1	31	+	*Bacteroides fragilis*	2+	+	<60
			Capnocytophaga sp.	$>10^5$		
2	32	−	*Streptococcus viridans*	$>10^5$	+	<60
3	33	−	Mixed anaerobic flora	−	NA	<60
			Proteus sp.	−		
4	36	−	*Ureaplasma urealyticum*	−	+	<60
5	24	+	*Escherichia coli*	$>10^5$	+	<60
			Citrobacter sp.	$>10^5$		
6	30	−	*Ureaplasma urealyticum*	−	NA	<60
7	31	−	*Ureaplasma urealyticum*	−	+	<60
8	34	−	*Ureaplasma urealyticum*	−	+	<60
9	29	+	*Gardnerella vaginalis*	$>10^5$	−	<60
10	34	−	*Mycoplasma hominis*	−	NA	484
			Ureaplasma urealyticum	−		
11	34	−	*Streptococcus agalactiae*	2000	+	200
12	29	−	*Mycoplasma hominis*	−	+	272
13	29	+	*Proteus mirabilis*	−	+	540
14	28	+	*Gardnella vaginalis*	−	+	658
15	32	−	Mixed anaerobic flora	−	+	1580
16	30	−	*Streptococcus agalactiae*	−	−	656
			Ureaplasma urealyticum	−		
17	33	−	*Ureaplasma urealyticum*	−	−	1632
18	22	−	*Bacteroides fragilis*	5000	NA	226
19	30	+	*Gardnella vaginalis*	−	NA	278
20	35	+	*Ureaplasma urealyticum*	−	+	1204
			Streptococcus viridans	$>10^5$		
21	33	+	*Mycoplasma hominis*	−	+	1804
			Ureaplasma urealyticum	−		
22	31	+	*Ureaplasma urealyticum*	−	−	1980
			Mixed anaerobic flora	>105		

NA, Not available.

during certain physiologic processes such as parturition.[14, 15]

This study was designed to determine the relationship between labor and microbial invasion of the amniotic cavity and the presence of TNF in amniotic fluid. The data obtained from patients with preterm labor (irrespective of membrane status) indicate that preterm parturition in the absence of microbial invasion of the amniotic cavity is not associated with the presence of detectable TNF in the amniotic fluid; only four of 39 patients intact membranes (Fig. 3) and none of 15 patients with premature rupture of membranes (Fig. 4) had detectable TNF in the amniotic fluid. Because one of these four patients had a positive Gram stain of amniotic fluid for microorganisms and another had an amniotic fluid white blood cell count of 3920 cells/mm^3 with 80% neutrophils, the possibility must be considered that these patients had microbial invasion of the amniotic cavity that escaped microbiologic detection.

On the other hand, our data indicate that there is a strong association between the presence of TNF in amniotic fluid and microbial invasion of the amniotic cavity. In each of the three populations studied (preterm labor and intact membranes, preterm premature rupture of membranes, and term labor), TNF was detected more frequently in women with a positive amniotic fluid culture for microorganisms than in women with a negative culture. Also, in the absence of microbial invasion of the amniotic cavity, preterm premature rupture of membranes was not associated with detectable TNF in the amniotic fluid regardless of the labor status at amniocentesis (Fig. 4). However, the rate of TNF detection in the amniotic fluid of women with microbial invasion of the amniotic cavity and labor was related to gestational age and membrane status. Women with preterm labor and intact membranes had a higher rate of detectable TNF than women with preterm premature rupture of membranes in labor or women with labor at term (92.3% [12/13], 59% [13/22], and 46.6% [7/15], respectively; $p < 0.05$) (Fig. 6).

The explanation for these findings is not readily apparent. We explored the possibility that differences in qualitative microbiology might be responsible for these observations. However, the proportion of patients with polymicrobial invasion of the amniotic cavity, positive

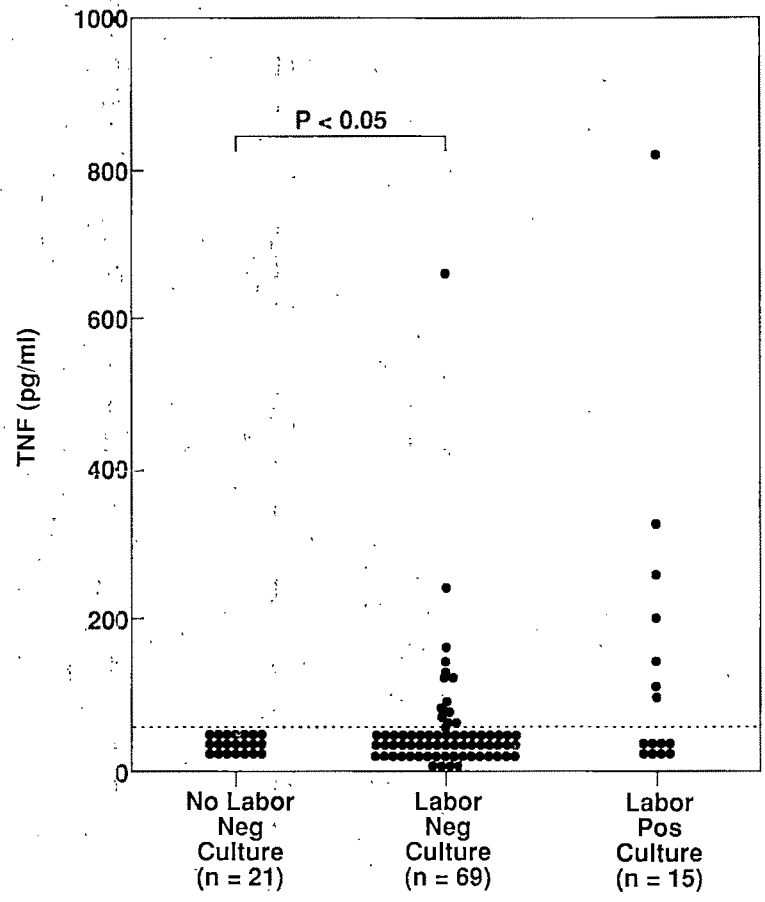


Fig. 5. Amniotic fluid TNF in women at term. TNF was detected more frequently in women in labor with microbial invasion of amniotic cavity than in women in labor without microbial invasion of amniotic cavity ($p < 0.05$). Also, TNF was detected more frequently in women in labor than in women without labor. ($p = 0.06$).

Table V. Amniotic fluid microbiologic and placental pathologic studies and amniotic fluid TNF concentrations in women with preterm premature rupture of membranes and microbial invasion of the amniotic cavity without preterm labor

Patient No.	*Gestational age (wk)*	*Gram stain*	*Culture*	*Quantitative microbiology*	*Histologic chorioamnionitis*	*TNF (pg/ml)*
1	31	–	*Peptostreptococcus*	–	NA	<60
2	34	–	*Mycoplasma hominis*	–	NA	<60
3	32	–	*Peptostreptococcus*	+1	+	<60
4	24	–	*Ureaplasma urealyticum*	–	+	<60
5	32	+	*Streptococcus viridans*	+1	+	<60
6	30.5	–	*Ureaplasma urealyticum*	–	+	<60
7	35.5	–	*Ureaplasma urealyticum*	–	NA	<60
8	26	–	*Lactobacillus sp.*	+1	NA	<60
9	20	–	*Ureaplasma urealyticum*	–	NA	<60
10	28	+	*Fusobacterium sp.*	60,000	–*	1163
11	33	–	*Ureaplasma urealyticum*	–	NA	464

NA, Not available.
*Acute deciduitis.

cultures limited to *Mycoplasma* or with gram-negative microorganisms was not different among these three groups. Although it is possible that the inoculum size might be different among groups, we were unable to test this hypothesis because the majority of patients did not have quantitative microbiologic studies performed.

An alternative view is that the detection rate of TNF in amniotic fluid is dependent on the pathway of microbial invasion and the time allowed for microbial-host interaction before amniocentesis.

Although the precise origin of amniotic fluid TNF has not been determined, intraamniotic and decidual

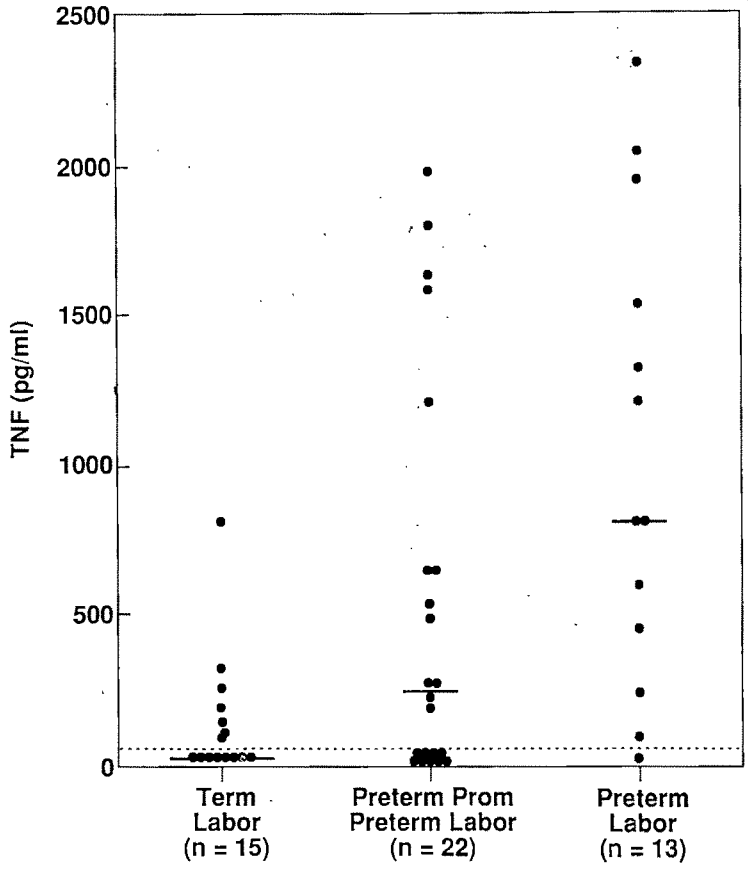


Fig. 6. Amniotic fluid TNF in women with microbial invasion of the amniotic cavity. TNF was detected more commonly in women with preterm labor and intact membranes than in women with term labor ($p < 0.05$). *Prom*, Premature rupture of membranes.

macrophages are potential sources. If the main sources are macrophages present in the amniotic cavity or fetal monocytes, the TNF concentration in amniotic fluid will be determined by factors regulating the state of monocyte or macrophage activation. Microbial invasion of the amniotic cavity of recent onset, low virulence, or limited inoculum size may lead to a modest macrophage response and TNF secretion into the amniotic fluid. This is the likely course of events associated with microbial invasion of the amniotic cavity that occurs in the context of spontaneous labor at term. In contrast, if microbial invasion of the amniotic cavity is of longer duration, of high virulence, or due to large inoculum size, macrophage activation will be more intense and amniotic fluid cytokine (including TNF) concentrations will be higher. This is the most likely course of events in cases of preterm labor associated with microbial invasion of the amniotic cavity. Another potential source of amniotic fluid TNF is decidual macrophages, which constitute approximately 30% of the total cell population of the decidua. However, we have no direct evidence, at this point, that TNF produced by the decidua can cross intact membranes into the amniotic cavity.

The role of decidual cytokine production in mediating parturition in the setting of infection probably varies with gestational age. In term pregnancy, mild decidual disruption such as that caused by stripping of the membranes or by a localized extraamniotic infection (deciduitis) may be sufficient to activate decidual macrophages to produce cytokines, stimulate myometrial contractility, and signal the onset of labor. In contrast, the magnitude of the stimuli (pharmacologic, mechanical, infectious-related) required to elicit parturition in early pregnancy seems to be considerably greater. This uterine unresponsiveness has probably evolved to protect against premature birth. Thus the earlier the gestation, the less likely that an extraamniotic infection by itself will be sufficient to signal parturition. In the absence of labor, microorganisms that have gained access to the decidua continue to proliferate, cross intact membranes, and then stimulate resident macrophages and other host cells (including fetal cells) to produce cytokines from within the amniotic cavity. This additional set of signals (to those generated during decidual infection) may then lead to parturition. If they do not, microorganisms may gain access to the fetus, activate fetal immunocompetent cells to produce cytokines, and elicit the endocrine changes associated with infection and parturition.

A puzzling finding in our study is that 20% of women in active labor at term with a negative amniotic fluid culture had detectable TNF in the amniotic fluid. Sev-

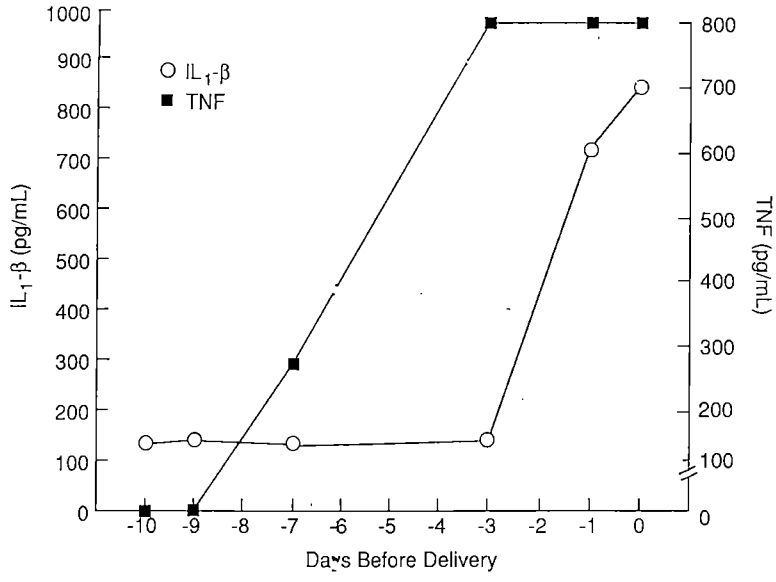


Fig. 7. Longitudinal observation of amniotic fluid TNF and interleukin-1β concentrations in patient in preterm labor with intact membranes and positive amniotic fluid culture.

eral explanations for this finding may be invoked. First, these patients may have had false-negative microbiologic studies. If this were the case, however, we would have to accept a rate of subclinical colonization of the amniotic cavity of 25% in term spontaneous labor. This estimate is higher than that reported by the only study using amniocentesis for collection of fluid in women in term labor.[16] An alternative view is that tissue disruption associated with parturition may lead to macrophage activation and the secretion of TNF in the amniotic cavity. This implies that a state of noninfectious macrophage activation may exist in some women with term spontaneous parturition. We have recently demonstrated that spontaneous parturition at term in the absence of demonstrable infection is associated with an elevation of amniotic fluid interleukin-6 levels.[12, 17] Because interleukin-6 can induce TNF gene expression by macrophages,[18] TNF detection in the amniotic cavity may be the consequence of activation of the cytokine network through a noninfectious mechanism. Further studies are required to clarify these issues. In situ hybridization analysis for cells containing TNF messenger ribonucleic acid and immunohistochemical localization of TNF protein in tissue sections are procedures currently available to address questions concerning the sources of amniotic fluid TNF and the relationship between inflammation, infection, parturition, and TNF expression.

Because TNF is capable of stimulating prostaglandin production by amnion[4] and decidua,[5, 6] we have proposed that this cytokine may participate in the signaling process responsible for the initiation of labor in the setting of infection.[4] Observations made in the current study of women with preterm premature rupture of membranes support this view. Indeed, the detection rate of TNF in amniotic fluid was greater in women with preterm labor and microbial invasion of the amniotic cavity than in women with microbial invasion but without preterm labor. These data indicate that parturition in the setting of infection is temporally associated with the secretion of TNF into the amniotic cavity and support the view that the initiation of parturition may be viewed as part of the repertoire of host-defense mechanisms elicited in response to intrauterine infection.

Further clinical support for this hypothesis is derived from longitudinal observations made in a patient in preterm labor (Fig. 7). This patient, admitted with preterm labor and intact membranes, had a positive amniotic fluid culture for *Candida* sp. The final episode of preterm labor and delivery, which occurred 11 days after admission, was preceded by the subsequent detection of amniotic fluid TNF and its increased concentrations in the amniotic cavity.

If preterm labor in the setting of infection is mediated by cytokines, including TNF, why then do 40.9% of women with preterm premature rupture of membranes and labor and a positive amniotic fluid culture not have detectable TNF? One possibility is that TNF is not required in all patients with infection to signal the onset of labor. Other members of the cytokine network, such as interleukin-1, may have a more critical role. Further studies in which several cytokines are assayed in the same sample are required to address this issue. Another possibility is that microbial invasion of the amniotic cavity in these patients occurred during the course of labor and that the mechanisms for initiation of parturition are unrelated to infection and cytokine. In these instances infection is the consequence rather than the cause of labor.

Can the assay of amniotic fluid TNF have clinical value? Although the current study was not specifically designed to address this question, the data from patients in preterm labor with intact membranes indicate that the detection of TNF in amniotic fluid in this population had a greater sensitivity than did the Gram stain in the diagnosis of microbial invasion of the amniotic cavity (sensitivity 92.3% [12/13] vs 61.5% [8/13], $p = 0.16$; specificity 89.7% [35/39] vs 97.4% [38/39], $p = 0.35$). Moreover, the presence of TNF in the amniotic fluid might identify a group of patients at risk for preterm delivery in spite of tocolytic treatment. Only one of the seven patients (14.2%) with detectable TNF and a negative Gram stain of amniotic fluid was delivered at term. It should be noted that this performance of amniotic fluid TNF determinations was not maintained in women with preterm premature rupture of membranes (TNF sensitivity 45.4% [15/33] and Gram stain sensitivity 33.3% [11/33], $p = 0.2$). If this observation is confirmed, the utility of TNF determinations in the diagnosis of microbial invasion of the amniotic cavity in patients with preterm premature rupture of membranes would be limited.

The use of cytokine determinations in the diagnosis of infection depends on the magnitude of the host response and not merely on the presence of microorganisms. This apparent limitation, however, could be exploited in clinical obstetrics. The combined use of modern microbiologic techniques (e.g., polymerase chain reaction) and cytokine determinations may identify the patient in whom microbial invasion is recent enough as to not have elicited a host response (i.e., cytokine secretion). This patient is likely to be the one who benefits the most from antibiotic treatment. Indeed, our data indicate that once microbial invasion of the amniotic cavity is accompanied by a robust cytokine secretion into the amniotic fluid, delivery is inevitable.

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LETTERS TO THE EDITORS

Magnesium sulfate is an unfit anticonvulsant in eclampsia

To the Editors: I read with great interest the article of Sibai (Sibai BM. Magnesium sulfate is the ideal anticonvulsant in preeclampsia-eclampsia. AM J OBSTET GYNECOL 1990;162:1141-5). As Sibai pointed out, magnesium sulfate has been recommended in the treatment of eclampsia; however, comprehensive data regarding its reliance and safety are lacking. I believe that patients will not be injured by the drug if the principles of treatment are followed.

There are multiple actions of magnesium sulfate that may be useful or nonhelpful to the fetus and mother. Effects on fetal heart variability and associated low Apgar scores, respiratory depression, hyporeflexia, and hypocalcemia have been reported in the neonates of mothers receiving intravenous magnesium sulfate. These effects were reported in premature infants in association with fetal growth retardation. Such complications may be present in these infants whether magnesium sulfate is or is not used. The perinatal mortality rate was reported to be as much as 17.1%[1] and 45%[2] when magnesium sulfate was used. Our hospital has not used magnesium sulfate, and the perinatal mortality rate is 2.78% (3/108).[3] In contrast, the maternal mortality rate in other large series of patients with eclampsia ranged between 3.3% and 14.4%, and the incidence of intracerebral hemorrhage was as high as 10.7% (Sibai's article). Dr. Sibai's patient was resuscitated and her seizures were controlled with magnesium sulfate. In our hospital taking care of patients with eclampsia without the use of magnesium sulfate has resulted in a maternal mortality of zero (0/106).[3]

Parenteral magnesium sulfate is the drug of choice to prevent convulsions in cases of eclampsia, but it is not suitable as an anticonvulsant in eclampsia. Magnesium sulfate controls convulsions of eclampsia but seriously endangers the health of both the mother and her fetus. We consider magnesium sulfate to be dangerous in our hospital; hence, it is not used.

Ji-qing Luan

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Reply

To the Editors: I do appreciate the interest of Dr. Luan in the subject of eclampsia and magnesium sulfate. Dr. Luan cites some statements from *my* manuscript without indicating the purpose or reason for including them, which makes it difficult to respond to his letter. He then cites a perinatal mortality rate of 2.78% in his experience with eclampsia as an indication not to use magnesium sulfate. He compares it to previous perinatal mortality reported in 1959 (his reference 2) and in 1979 (his reference 1). Again, I fail to see the association since he does not explain what is meant by perinatal mortality. Did he include patients who had stillbirths or neonatal deaths before arrival to the hospital? What are the gestational ages? Did he include patients with gestational ages <28 weeks? Currently, neonatal survival for infants >28 weeks' gestation is almost 100% in Memphis. This has nothing to do with magnesium sulfate. In addition, neonatal survival for preterm babies in Memphis is definitely superior to that in China. He also mentions maternal mortality without use of magnesium sulfate. Again, he does not mention the condition of the patient on admission and how many were excluded before admission.

Finally, he concludes that magnesium sulfate is an unfit anticonvulsant in eclampsia. It is surprising for someone who has never used the drug to make such a statement. In addition, he does not report what drug he uses. It is ridiculous to make such statements without data to support them.

Baha M. Sibai, MD

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The issue of animal rights and human rights

To the Editors: I agree with much of what Mister Maharry (Maharry TM. The issue of animal rights and human rights. AM J OBSTET GYNECOL 1991;164:1543-8) says about medical research being dependent on animal experimentation. I support the use of animals in appropriately screened experiments. I am also a member of the Physicians Committee for Responsible Medicine, and I believe that there are substantial experiments going on in this country that use animals. A number of these experiments are superfluous or do not particularly further the cause of medical knowledge. I believe this type of experiment still needs to be weeded out. Although some animal activist groups have been violent, I believe that in general the cause for protection against cruelty to animals has been advanced. We have all become more sensitive to the injustices that humans have dealt the animal world.

I cannot deny that violence is not an appropriate response, but as an animal rights advocate I believe closer scrutiny of the use of animals in experimentation and closer scrutiny of the usefulness of the experiments themselves is an appropriate response. Do not lump all animal rights activists with the few violent ones. We need to take a close look at our respect for the animal kingdom. Many species have been destroyed through

our carelessness. Perhaps being made more sensitive to their plight will benefit humankind in a way more significant than it may seem at face value. Food for the hungry is important, but survival of the planet to feed all of us is most important.

Fredericka Heller, MD
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Response declined

Fetal biophysical and umbilical cord gases

To the Editors: The article by Vintzileos et al. (Vintzileos AM, Fleming AD, Scorza WE, et al. relationship between fetal biophysical activities and umbilical cord blood gas values Am Obstet Gynecol 1991;165:707-13) is an important contribution to our understanding of some aspects of fetal pathophysiology and its detection before significant morbidity or fetal death. I have some observations about this paper.

First, the title of Table VI should be Umbilical venous blood rather tham Umbilical artery blood.

Second, the authors did not comment on the influence, if any, of the maternal oxygen administration rate of 3 L/min on umbilical cord venous and artery Po_2. In spite of maternal oxygen administration the values of umbilical venous and artery Po_2 with reactive nonstress test and fetal breathing movements present appear to be low. Would a contraction stress test have been more useful in identifying impending fetal-neonatal morbidity at a higher value of venous and artery Po_2?

Third, it is not surprising to note that fetal hypercapnia occurred only when fetal movement and tone were absent and was associated with significant fetal acidosis and hypoxia. This occurrence is explained by the physiologic fact that Co_2 exchange across placenta membranes or alveoli is much more efficient than the exchange of oxygen.

Houchang Modanlou, MD
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Reply

To the Editors: We thank Modanlou for his interest in our work. We agree with his first comment that the title of Table VI should be umbilical venous blood rather than umbilical artery blood.

In our opinion the relatively low umbilical cord venous and artery Po_2 in spite of maternal oxygen administration of 3 L/min was related to the fact that the oxygen administration was of very short duration. This time period was from satisfactory epidural analgesia (when skin incision was made) until delivery of the infant. However, the main emphasis of our study was to investigate the relationship between the presence or absence of the individual fetal biophysical activities and umbilical cord blood gas measurements and to compare the different levels of pH, Po_2, Pco_2, and bicarbonate and base excess at which the individual biophysical activities become compromised. We did not attempt to establish normal umbilical cord venous and artery Po_2 values. Therefore for reasons of comparison the absolute Po_2 levels were not important because all patients received epidural analgesia and had the same degree of maternal oxygenation. We doubt that a contraction stress test could be useful in identifying impending fetal-neonatal morbidity. In general the appearance of late decelerations of the fetal heart rate in response to uterine contractions may suggest fetal hypoxia caused by uteroplacental insufficiency. However, this concept is valid only during the intrapartum period because the frequency and intensity of the uterine contractions can be monitored with internal monitoring techniques. External monitoring techniques like those used in the antepartum contraction stress test, however, are not capable of measuring the intensity of the uterine contractions and therefore the strength of the stimulus to the fetus. In our opinion any judgment about the presence or absence of fetal hypoxia when the intensity of the stress factor (uterine activity) is unknown is not reasonable and has no scientific basis.

We agree with Modanlou's comment that carbon dioxide exchange across placental membranes is much more efficient that oxygen exchange. We also believe that fetal hypercapnia in the absence of fetal movement and tone may also be related to umbilical cord compression, as is frequently seen in cases in which oligohydramnios is associated with intrauterine growth retardation and compromised biophysical activities.

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Hemolytic disease of the fetus

To the Editors: Weiner et al. (Weiner CP, Williamson RA, Wenstrom KD, et al. Management of fetal hemolytic disease by cordocentesis. I. Prediction of fetal anemia. Am J Obstet Gynecol 1991;165:546-53) report a management scheme for hemolytic disease of the fetus based on results from cordocentesis. Although the authors stop short of recommending the abandonment of amniocentesis, it is clear that in their unit they have. Although their results were good, there was no prospective comparison to an amniocentesis-based management scheme. Unfortunately, the authors (and readers) can only speculate on the broad range of relative advantages and disadvantages of each scheme.

My questions regard the selection of patients for invasive testing. The authors state that an indirect Coombs' titer of $\geq$1:8 was selected for testing because in their unit severe hemolytic disease has occurred at 1:8 but not below. This same threshold was reported by Reece et al.[1]; it places the critical titer for their laboratories at 1:4, which is one dilution stricter than that recommended by the American College of Obstetricians and Gynecologists Technical Bulletin[2] (>1:8) and two dilutions stricter than the data-based recommen-

dations of Freda[3] and Queenan[4] (>1:16). As a consequence of this stricter critical titer, more patients require invasive testing or require it earlier in pregnancy.

My questions: (1) In their current series, in a first sensitized pregnancy what was the lowest fetal hematocrit observed when the indirect Coombs' titer within 1 to 2 weeks of sampling was ≤1:16? (2) Do the observations in the current series justify invasive sampling in the first sensitized pregnancy when a recent indirect Coombs' titer is 1:8 or 1:16? (3) Can their historic observation of severe fetal hemolytic disease with a titer of 1:8 be explained by (a) its occurrence in an other-than-first sensitized pregnancy, (b) delivery remote from the 1:8 titer with possible intervening unobserved increases in titer, or (c) an unobserved increase in titer caused by invasive sampling after the titer? MacGregor et al.[5] reported a rise from 1:8 to 1:4096 within weeks of cordocentesis.

If the answers to these questions do not support invasive testing in first sensitized pregnancies with 1:8 or 1:16 titers, the authors should acknowledge this and adjust their critical titer. This adjustment would allow the selection of invasive testing only when the fetal risk truly warrants it.

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REFERENCES

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3. Freda JF. The Rh problem in obstetrics and a new concept of its management using amniocentesis and spectrophotometric scanning of amniotic fluid. AM J OBSTET GYNECOL 1965;92:341-74.
4. Queenan JT. Modern management of the Rh problem. 2nd ed. Hagerstown, Maryland: Harper & Row, 1977: 31-3.
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Reply

To the Editors: We thank Spinnato for his inquiry. We expect may questions will arise regarding the proposed management scheme because it is difficult to give adequate detail in an initial publication.

Spinnato asked about the antibody titer at which an invasive evaluation should be undertaken during the first sensitized pregnancy. His specific concern was that we selected 1:8 although the American College of Obstetricians and Gynecologists Technical Bulletin states that a titer of <1:16 indicates that the fetus is not in serious jeopardy. His question is best answered by quoting directly from that technical bulletin. "In general, it is believed that, if the antibody titers remain less than 1:16 in an initial immunized gestation, the fetus is not in serious jeopardy. *It is important to know, however, that this value is somewhat dependent on local laboratory methods, skill of laboratory personnel, and previous experiences related to titers within that institution* [emphasis added]."

Spinnato is not the first to have missed this point. With the decreasing incidence of fetal hemolytic disease, experience with the entity has shrunk. Our critical titer has been and remains 1:8 and is the result of many years of experience in one laboratory with a wide referral area. All physicians involved in the evaluation of fetal hemolytic disease should find the critical titer in their individual laboratories. Obviously this is impossible in a small hospital; regionalization is required.

In our experience patients have frequently been referred with a low indirect Coombs' titer from the local laboratory, only to have a much higher titer when tested by the University of Iowa Hospitals and Clinics blood bank. Whether this reflects a change in sensitization or differences in laboratories, I cannot state.

Our data base does not include information as to whether the pregnancy under evaluation was the first sensitized pregnancy. As a rule we monitored each patient during her first sensitized pregnancy with serial indirect Coombs' titers. We require a titer ≥1:8. Ten nulliparous women have undergone cordocentesis for red blood cell isoimmunization. Two of those fetuses had anemia when first evaluated. In one, sensitization was to D and the indirect Coombs' titer was 1:1024. In the other, sensitization was to Kell and the indirect Coombs' titer was 1:8. However, most of the patients who have required transfusion with low indirect Coombs' titers were not in their first sensitized pregnancies. Thus our observation of severe hemolytic disease with a titer of 1:8 is mostly, but not exclusively, explained by its occurrence in a subsequent sensitized pregnancy. As Spinnato notes, other have had similar experiences with their laboratories.

Spinnato refers to a case report by MacGregor et al., who observed an abrupt increase in antibody titer within weeks of cordocentesis. However, I note that the fetus in question was already anemic when the titer was 1:8. This raises an important point. Cordocentesis is but a procedure. One must not only know what the normal values are, but what to do with them. With our management scheme we have tried to supply a course of action. The procedure can also be misused. To prevent worsening of disease we avoid the placenta in patients with low-grade sensitization by sampling from a midsegment.

In summary, the 1:16 guideline in the American College of Obstetricians and Gynecologists Technical Bulletin was never intended to be engraved in stone. It is clear that at the University of Iowa our critical titer is 1:8. Our experience is that these fetuses are at risk and benefit from accurate testing.

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Ultrasonographic screening for trisomy 21

To the Editors: In the article by Lockwood et al. (Lockwood CJ, Lynch L, Berkowitz RL. Ultrasonographic screening for the Down syndrome fetus. AM J OBSTET GYNECOL 1991;165:349-52) the authors estimate the positive predictive value for Down syndrome of both the biparietal diameter/femur length ratio and measured femur length/expected femur length ratio for a given biparietal diameter. To calculate this predictive value they used a prevalance of Down syndrome (1/710) that reflects the incidence of Down syndrome at 16 weeks' gestation for the general population. An alternative estimate of the prevalence in a target population might include all pregnancies in women <35 years of age and 50% of woman >34 years who do not have amniocentesis. This formula would lower the prevalence (assuming there are 4.2 million live births per year[1], that 8.6% of live births occur to women >34 years of age, that 39% of Down syndrome births occur in women >34 years,[2] and that Down syndrome occurs in 1/1000 live births) to 1/884 at 16 weeks and reduce the positive predictive value to below the levels cited by approximately 20% to 30%.

Additionally, there appears to be an error in the estimate of predictive value for study 9 in Table I. The correct positive predictive value should be 1/108 rather than 1/644.

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REFERENCES

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2. Goodwin BA, Huether CA. Revised estimates and projections of Down syndrome births in the United States, and the effects of prenatal diagnosis utilization, 1970-2002. Prenat Diagn 1987;7:342-6.

Reply

To the Editors: We thank Bernstein for his analysis of the positive predictive value of the various biparietal diameter/femur length formulas for detecting fetal Down syndrome. He is correct in his analysis of this alternate approach to the estimation of positive predictive values. In addition, he rightly points out a typographic error in study 9. The positive predictive value of this study is indeed 1/108 rather than 1/644.

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What is the prognosis for congenital cytomegalovirus infection?

To the Editors: We read with interest the article by Lynch et al. (Lynch L, Daffos F, Emanuel D, et al. Prenatal diagnosis of fetal cytomegalovirus infection. AM J OBSTET GYNECOL 1991;165:714-8). This article demonstrates the usefulness of a comprehensive evaluation of fetuses with possible congenital cytmegalovirus infection. Although the numbers are small (6 of 7), it appears that women who have a documented primary infection during pregnancy can be reassured if their fetus has normal blood studies, normal ultrasonographic findings, and a negative amniotic fluid culture.

Although we agree that the prognosis is likely to be poor for congenitally infected infants who are detected solely on the basis of abnormal ultrasonographic findings, this is not certain. There has been at least one case in which ascites diagnosed at 27 weeks' gestation completely resolved before birth at 36 weeks' gestation.[1] This infant had minimal findings at birth.

The natural history of congenital cytomegalovirus infection is still not known in detail. We agree that more long-term follow-up of fetuses and neonates known to be infected in utero is needed.

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REFERENCE

1. Binder ND, Buckmaster JW, Benda GI. Outcome for a fetus with ascites and cytomegalovirus infection. Pediatrics 1988;82:100-3.

Response declined

Omental graft made simple

To the Editors: Since my article (Petty WM, Lowy RO, Oyama AA. Total abdominal hysterectomy after radiation therapy for cervical cancer: use of omental graft for fistula prevention. AM J OBSTET GYNECOL 1986;154:1222-6) was published, it has been found that it is not usually necessary to make a pedicle graft of the omentum to attach it to the vagina. Normally the omentum is long enough to reach the vaginal apex without being on tension. If the omentum will reach the vaginal apex without being on tension, it can be gently sutured to the vaginal apex with two interrupted absorbable sutures after the vagina is closed. I have only had to make a pedicle graft once in approximately 12 additional cases. The other 11 times the omentum has reached the vagina without being on tension. Therefore a simplified method of preventing fistulas after either full radiation therapy or a partial radiation therapy with hysterectomy is to simply suture the distal omentum to the vaginal apex. It is quick, easy, takes only 2 minutes, and has completely prevented fistulas in my patients.

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Ovarian cancer screening

To the Editors: The issue of screening for ovarian cancer is a timely one: it is of particular concern not only to

gynecologists and other primary care physicians, but also to imaging specialists. It is unfortunate, therefore, that in the otherwise excellent review of this topic by Creasman and DiSaia (Creasman WT, DiSaia PJ. Screening in ovarian cancer. AM J OBSTET GYNECOL 1991;165:7-10) the authors based their opinion of ultrasonography as a screening technique on three ultrasonography articles from 1988 and 1989 (reflecting the standard of imaging of the years preceding publication). None of the referenced articles included transvaginal evaluation of the ovaries or Doppler visualization of the supplying ovarian and uterine arteries. This omission is particularly distressing in that the same issue of the journal that contained the ultrasonography article by Campbell et al.[1] also contained another paper[2] in which that group reported their early and encouraging results with the use of transvaginal color flow imaging to identify malignant ovarian masses.

Since that time multiple papers have appeared on the use of transvaginal evaluation of ovarian masses and the characterization of the blood flow by Doppler technique. Our own recently published work[3] has demonstrated the use of transvaginal ultrasonography in identifying very small, nonpalpable masses in asymptomatic postmenopausal women.

Because of the tremendous advances that have been made in ultrasonographic technology within the past 2 years, I believe that basing such an important conclusion on outmoded technology is a disservice not only to your readers, but also to the millions of women who are concerned about their risk for ovarian carcinoma. There is no doubt that a large multicenter study is needed to evaluate the use of modern diagnostic ultrasonography in the identification of early ovarian carcinoma. Campbell and Bourne and their colleagues have made a good start. Meanwhile it is counterproductive to deny our patients such a valuable imaging study while waiting the 5 to 10 years it will take for final results.

Barbara B. Gosink, MD

Department of Radiology, University of California, San Diego, Medical Center, 225 Dickinson St., San Diego, CA 92103-1990

REFERENCES

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2. Bourne T, Campbell S, Steer C. Whitehead MI, Collins WP. Transvaginal colour flow imaging: a possible new screening technique for ovarian cancer. BMJ 1989;299: 1367-70.
3. Wolf SI, Gosink BB, Feldesman MR, et al. Prevalence of simple adnexal cysts in postmenopausal women. Radiology 1991;180:65-71.

Reply

To the Editors: We appreciate Gosink's interest in our article. Her main criticism is that we quoted studies using supposedly antiguated technology (abdominal ultrasonography). The studies we referenced were published in 1988 and 1989—not necessarily ancient history. We are well aware of transvaginal ultrasonography and color flow Doppler. The ability of transvaginal ultrasonography in regard to sensitivity in identifying ovarian follicles is well established with the in vitro fertilization programs. We chose those articles because they were by far the best and largest studies published. There are no like published articles with transvaginal ultrasonography as a screening tool for ultrasonography! Gosink noted that Campbell et al. had published an article on color flow Doppler and wondered why it was not referenced in our publication. That study is a presentation of 50 *selected* patients and does not represent a technique or data on screening for ovarian cancer.

Gosink suggested that transvaginal ultrasonography is better technology than abdominal ultrasonography and quoted one of their own articles. In their article both abdominal and transvaginal ultrasonography were used in 149 patients. They actually identified more ovaries with the abdominal ultrasonography than the transvaginal method (58% vs ≤1%). In fact, they were unable to identify 32% of the ovaries known to be present with either technique. Their inference was that if they did not see the ovaries, cysts were probably not present (the main thrust of their paper was the identification of "cysts" in a postmenopausal patient). On the other hand, Campbell has made the point that it is necessary to identify the ovary to state that no pathologic condition is present (their report was based on over 30,000 ovaries). Gosink stated that 30 minutes was the usual time for ultrasonographic evaluation, with the radiologist involved some of the time. We wonder what the cost would be if this were not a study?

One of the points in our article was that the incidence of ovarian cancer (approximately 20,000 per year) is relatively low, particularly when compared with other cancers (breast—approximately 175,000 per year); therefore any screening test is probably not going to be effective in making a major impact on the dismal survival rate for this cancer. As noted from the paper by Einhorn et al.[1] quoted in our article, the CA 125 test was used as a screening test. Einhorn et al. stated that considering the incidence of ovarian cancer in Sweden, even if the test had a specificity of 99.5%, only 6% to 11% patients with elevated CA 125 levels would actually be expected to have cancer even if the sensitivity was 100%. In Campbell's study, for every 67 patients coming to surgery because of abnormality noted on ultrasonography examination, only 1 was expected to have a primary ovarian cancer, obviously a very high false-positive rate. After their large study with over 30,000 ovaries evaluated, Campbell et al. have stated that the cost-effectiveness of ultrasonography must now be determined. There are several ongoing studies evaluating this technique alone and with tumor markers. Even if transvaginal is proved to be better than abdominal

(which it has not), is it still cost-effective as a screening technique?

To the statement that new technology makes previous data (abdominal ultrasonography) no longer applicable, we argue that it is not appropriate to accept new technology carte blanche without data supporting its effectiveness. Even if transvaginal ultrasonography were 100% sensitive and specific, an accuracy rate no one suggests, there would still be major problems because of the numbers. To take new technology without proving its effectiveness as state of the art may add tremendous expense to already massive health care costs without decreasing the mortality of this cancer and is a disservice to our patients and medicine in general.

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Philip J. DiSaia, MD

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REFERENCE

1. Einhorn N, Sjovall K, Schoenfeld G, et al. Am Soc Clin Oncol 1990;90:157.

Epidermal growth factor receptor expression in cervical intraepithelial neoplasia

To the Editors: I read with interest a recent article by McGlennen et al. (McGlennen RC, Ostrow RS, Carson LF, Stanley MS, Faras AJ. Expression of cytokine receptors and markers of differentiation in human papillomavirus–infected cervical tissues. AM J OBSTET GYNECOL 1991;165:696-705). The authors found increased levels of epidermal growth factor receptor with increasing levels of cervical dysplasia. In our study[1] we found levels of expression of messenger ribonucleic acid for epidermal growth factor receptor to correlate with levels of cervical dysplasia, with expression of epidermal growth factor receptor messenger ribonucleic acid in basal and parabasal epithelium in normal cervix, and up to and above midepithelium in moderate and severe dysplasia. Presence of messenger ribonucleic acid for epidermal growth factor receptor suggests not only presence but continued synthesis of epidermal growth factor receptor in dysplastic cells. The study by McGlennen et al. shows actual increase in the amount of epidermal growth factor receptor with increasing severity of dysplasia, a finding that further supports a role for epidermal growth factor receptor in the pathogenesis of cervical dysplasia.

Fig. 3 and its legend in the article by McGlennen et al. are mislabeled as showing *EGF* (epidermal growth factor) instead of *EGFR* (epidermal growth factor receptor).

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REFERENCE

1. Mittal K, Pearson J, Demopoulos R. Patterns of mRNA for epidermal growth factor receptor and keratin B-2 in normal cervical epithelium and in cervical intraepithelial neoplasia. Gynecol Oncol 1990;38:224-9.

Response declined

ITEMS

Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $60.00 U.S. per insertion and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to Kay G. Goehler, Senior Manuscript Editor, Journal Editing, Mosby–Year Book, Inc., 11830 Westline Industrial Drive, St. Louis, MO 63146-3318.

Advances in Urogynecology and Pelvic Surgery, Sheraton Chicago Hotel, Chicago, Illinois, June 25-27, 1992. Sponsored by the Section of Urogynecology, Evanston Hospital. For information, contact the Office of Continuing Medical Education, Northwestern University Medical School, 303 East Chicago Ave., Ward 1-004, Chicago, IL 60611-3008. Tel.: (312) 503-8533.

5th Congress of the International Perinatal Doppler Society, New York City, Waldorf Astoria Hotel, August 29–September 2, 1992. For information contact: Professional Planners, Ltd., 3700 El Prado Blvd., Coconut Grove, FL 33133. Tel.: (305) 443-6728.

Hands-on Workshop in Reproductive Ultrasound and Prematurity Prevention, August 1-2, 1992, Marriott's Seaview Resort, Atlantic City, New Jersey. AMA Category I credit. For further information contact: Michele Gambino, Pennsylvania Hospital, Department OB/GYN, 822 Pine St., Suite 1A, Philadelphia, PA 19107. Tel.: (215) 829-3611.

The Harvard Medical School, Department of Continuing Education, and The Massachusetts General Hospital, Department of Gynecology, are presenting a postgraduate course in: Vaginal Surgery, directed by David H. Nichols, MD, on October 9, 10, 11, 1992, at The Ritz-Carlton Hotel, Boston, Massachusetts. For information contact: Harvard Medical School, Department of Continuing Education. Tel.: (617) 432-1525.

Bound volumes available to subscribers

Bound volumes of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY are available to subscribers (only) for the 1992 issues from the Publisher, at a cost of $68.00 for domestic, $95.76 for Canada, and $91.00 for international for Vol. 166 (January-June) and Vol. 167 (July-December). Shipping charges are included. Each bound volume contains a subject and author index and all advertising is removed. Copies are shipped within 60 days after publication of the last issue in the volume. The binding is durable buckram with the JOURNAL name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact Mosby–Year Book, Inc., Subscription Services, 11830 Westline Industrial Drive, St. Louis, MO 63146-3318, USA; phone (800) 325-4177, ext. 4351, or (314) 453-4351.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular JOURNAL subscription.

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The Women's Pavilion of The Norton Hospital, part of Alliant® Health System in Louisville, KY, is a recognized leader in providing maternal and newborn care. In conjuction with the University of Louisville, The Women's Pavilion is currently recruiting a seasoned Director to help meet the increasing demands of IVF procedures.

A team of reproductive endocrinologists and other medical professionals experienced in infertility, biochemistry, and embryology direct the nationally recognized Fertility Program at The Women's Pavilion. The LABORATORY DIRECTOR will be responsible for all standard IVF procedures as well as sperm/egg micro manipulation, embryo freezing and storage, and egg donation. This position also ensures the embryogenic process and the availability of reliable lab results and procedures; educates patients, physicians and other healthcare personnel; and provides interpretation of statistical data.

Qualified candidates will possess a Ph.D. in Embryology and two years of embryology and androilogy experience in an in vitro fertilization program.

Alliant Health System offers a competitive salary and comprehensive benefits package including relocation assistance.

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Nancy Borgstrom
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1020 Bandana Boulevard West
St. Paul, MN 55108
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Equal Opportunity Employer

The Department of Obstetrics and Gynecology at Boston University Medical Center and Boston City Hospital is seeking a:

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for expanded services in our department and affiliated community hospitals. Responsibilities would include clinical care, teaching and research. Academic rank and salary will be dependent upon the training, credentials and experience of the applicant.

Please send inquiry and curriculum vitae to: David Acker, M.D., Chairman, Department of Obstetrics and Gynecology, Boston University School of Medicine, 80 East Concord Street, Boston, MA 02118. Boston City Hospital and Boston University Medical Center are equal opportunity employers.

Boston University Medical Campus

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Division Chief, Ambulatory OB/GYN Programs

The Department of Obstetrics, Gynecology, and Reproductive Biology at Brigham and Women's Hospital, a major teaching hospital of Harvard Medical School, seeks a full-time Obstetrician/Gynecologist to direct its ambulatory care programs.

Strong interpersonal and management skills, as well as commitment to clinical care, quality improvement, and teaching are essential.

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Applicants must be Board-certified in Obstetrics and Gynecology, and must have clinical experience in a large, academic center.

Academic rank at Harvard Medical School will be commensurate with experience, training, and achievements. Send inquiry and curriculum vitae to:

Kenneth J. Ryan, M.D.
Chairman, Department of OB/GYN
Brigham and Women's Hospital
75 Francis Street, ASB1-3-073
Boston, MA 02115

The Brigham and Women's Hospital is an affirmative action, equal opportunity employer.

BRONSON

Obstetrician/Gynecologist Opportunity Available
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A practice opportunity is available for BE/BC Ob/Gyns t join a well-established, busy hospital-based group practice. Excellent salary and malpractice insurance are among the many benefits of this opportunity. Relocatior and interviewing expenses will also be provided.

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Bronson Hospital, the 442-bed tertiary care facility for southwestern Michigan, has a 500-member medical sta which includes 31 Ob/Gyns, 3 perinatologists and 6 neonatologists. An average of 3,400 deliveries are performed annually at Bronson.

Kalamazoo, located midway between Chicago and Detroit, offers diverse cultural opportunities. Lake Michigan's sandy beaches and dunes are only minutes away and numerous lakes, hills and forests provide boundless recreational activities. Excellent schools, several colleges and a major university further contribut to a high quality life-style. Real estate is affordable and one can be in the beautiful countryside within 10 minutes.

For more information about this practice opportunity, please call or send CV to:

Debra Hartman, Bronson Administrative Services
252 E. Lovell St., Kalamazoo, Michigan 49007
(800) 594-9022 or (616) 341-6331

DIRECTOR
OBSTETRICS AND GYNECOLOGY
Broadlawns Medical Center
Des Moines, Iowa

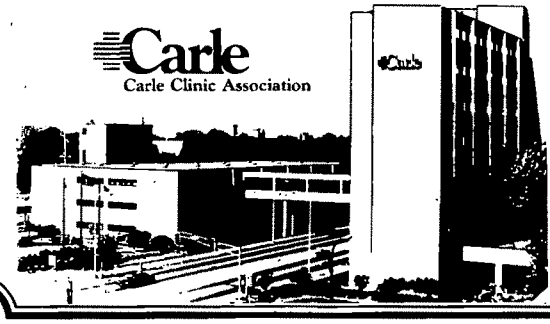
The University of Iowa and Broadlawns Medical Center, a 200+ bed county supported public teaching hospital, are seeking a Director for the Department of OB/GYN. Activities will include supervising patient care as well as teaching of Family Practice residents, a rotating OB/GYN Resident, and medical students in our OB service (500 births per year and growing). Department includes medical office clinic facilities, a new Family Birthing Center, a Family Planning Program and a newly created mid-wife position. Basic requirements include an M.D. or D.O. degree, and Board Certification or active candidacy of the American Board of Obstetrics and Gynecology, extensive practice experience, and the ability to direct staff and programs to support the service and educational goals of the institution. Clinical teaching experience would be desirable. A university medical school faculty appointment is possible for appropriate candidates. If interested, contact D.J. Walter, MD, Director of Medical Education, Broadlawns Medical Center, 18th & Hickman Road, Des Moines, Iowa, (515) 282-2203. Minorities and women especially encouraged to apply. The University and Broadlawns are Equal Opportunity/Affirmative Action Employers.

OBSTETRICIANS AND GYNECOLOGISTS
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OB/GYN Physicians needed to join Carle Clinic Association, a 225 physician, multi-specialty group practice in central Illinois with opportunities at the main campus as well as three additional sites - Urbana, Bloomington, Danville and Mattoon/Charleston. These communities offer recreational, cultural and educational amenities. Benefit package includes a competitive salary with partnership and income sharing plan after two years, professional liability insurance, health, life and disability insurance and generous vacation and meeting time. Contact:

Robert C. Parker, Jr., MD
Medical Director
Carle Clinic Association
602 W. University, Urbana, Illinois 61801
Phone 217-383-3399 - Fax 217-383-3163

Carle
Carle Clinic Association



FACULTY POSITIONS
GENERALIST OBSTETRICIAN/ GYNECOLOGIST
MATERNAL/FETAL MEDICINE SUBSPECIALIST
GYNECOLOGIC ONCOLOGIST
REPRODUCTIVE ENDOCRINOLOGIST

The James H. Quillen College of Medicine of East Tennessee State University offers an excellent opportunity to physicians of these specialties who are Board Certified or Board Eligible. This opportunity offers both tangible and intangible benefits sometimes not found in larger cities. Our emphasis is on excellence in service to patients, education, and research. These Faculty positions, both Tenure track and Grant-supported, are at the Assistant/Associate Professor level and offer very competitive salaries and excellent fringe benefits. The Gynecologic Oncologist position is funded by an outside source. The Tri-Cities area boasts of good schools and suburban living in beautiful Northeast Tennessee with easy access to various cultural activities. Our outdoor sports include all water sports, snow skiing, the Appalachian Trail, and numerous scenic attractions. Our medical referral base consists of 1.3 million people within a 50 miles radius. Send curriculum vitae to:

Frederick R. Jelovsek, M.D.
Professor and Chairman,
Dept. of OB/GYN
James H. Quillen College
of Medicine
East Tennessee State University
Box 70,569
Johnson City, TN 37614-0569

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Perinatologist Needed in Coastal New Jersey

2 member group is seeking a third perinatologist (BE/BC). We are active practitioners of perinatology and we also serve as integral members of the teaching team for a residency in Obstetrics and Gynecology.

We engage in both in-patient and out-patient activities, and all skills related to maternal fetal medicine may be used within our practice. Strong ultrasound skills are required, and the current practice consists of antenatal diagnosis, genetic counseling, amniocentesis, CVS and PUBS. We will insist on comfort and competence in basic obstetrical practice.

We are located about 1.5 hours from both New York City and Philadelphia, and the area provides a suburban life style.

Contact: Glenn Herman, M.D.
David Wallace, M.D.
Telephone: (908) 870-3600
Fax: (908) 870-0119

MATERNAL/FETAL SUBSPECIALISTS
EASTERN PENNSYLVANIA

Lehigh Valley Hospital, an 830-bed tertiary care teaching hospital, is seeking additional faculty including two full-time maternal/fetal medicine subspecialists: a division chief and an associate. The physicians' roles will include consultative patient care, program development, student and resident education and research if desired. The opportunity exists to customize the role to your particular career goals. Candidates should be board certified or active candidates for certification in maternal/fetal medicine.

Lehigh Valley Hospital is a regional Perinatal Center with over 3,300 births annually, of which 40 percent are high-risk. The department is supported by a 20-bed Level III NICU and four full-time neonatologists. The hospital serves as a clinical campus of Hahnemann University and maintains a free-standing, fully-accredited OB/GYN residency program.

Allentown, Pennsylvania is a cosmopolitan city, 60 miles north of Philadelphia and 80 miles west of New York City. The area has excellent school systems, nine colleges and universities and diverse cultural offerings. An excellent compensation package has been designed to recruit qualified candidates. Please send CV to **Carol Voorhees, Vice President, Lehigh Valley Hospital, 1243 S. Cedar Crest Boulevard, Suite A, Allentown, PA 18103, or call (215) 821-3090.** EOE.

PERINATOLOGIST
OGDEN, UTAH

An outstanding opportunity exists for a perinatologist to set up a consultative practice in Ogden, Utah. The physician will be an employee of McKay-Dee Hospital, a 415-bec community facility. Office space, personnel and equipment will be provided by the hospital. The successful candidate will be an integral part of Intermountain Health Care's Perinatal Group.

Ogden, Utah is a beautiful community of 69,000, with a service population of 260,000. There is an abundance of outdoor recreation available, including skiing, snowmobiling, hunting, fishing, boating, swimming and camping. A variety of cultural events entertain and amuse Ogden residents, such as the symphony, the ballet, museums, art galleries and festivals. Ogden is a short 40 minutes from Salt Lake City where more professional sports and cultural events may be found.

For more information, send a CV to:
IHC Physician Placement Services
P.O. Box 57010
Murray, UT 84157-0010
or call toll-free, 1-800-888-3134

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115 Perimeter Center Place
Suite 380 11807
Atlanta, GA 30346
Tel. 1-800-544-1987

JACKSON AND COKER

Harvard Medical School and mount Auburn Hospital are jointly seeking a Chief of Obstetrics and Gynecology at Mount Auburn Hospital whose primary interest is in Obstetrics. He/she will direct and expand an active clinical program, develop a residency program, teach medical and graduate students in the specialty, will hold academic appointment at Harvard Medical School commensurate with training and experience, and will also have an appointment at the Massachusetts General Hospital. He/she will be encouraged to develop an academic program of research in his/her respective field. Salary support will be provided by Mount Auburn Hospital and Harvard Medical School. Please send curriculum vitae to: Isaac Schiff, M.D., Chief of Gynecology, Massachusetts General Hospital, Vincent-1, Boston, MA 02114. Telephone 617-726-3001. Mount Auburn Hospital, Harvard Medical School and Massachusetts General Hospital are Equal Opportunity Employers.

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Excellent opportunity for Board Certified or Eligible OB/GYN Physician to join twenty-six physician, well established multi-specialty group. Position available immediately with full practice due to recent relocation of one of the two associates in the OB/GYN Department. Will join a well established Board Certified OB/GYN physician. Good starting salary with an incentive program. Excellent corporate fringe benefit program. Practice situated in medium size city 45 miles north of Denver, Colorado, in the beautiful front range of the Rockies.

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Administration
Longmont Clinic, P.C.
1925 West Mountain View Ave.
Longmont, CO 80501
(303) 776-1234 (collect)

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Challenging practice that admits to a tertiary teaching hospital with a fully accredited OB/GYN residency program

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A 1-in-5 call schedule with predictable time off

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PERINATOLOGIST MINNEAPOLIS

North Memorial Perinatal Center is seeking a board eligible/board certified Maternal Fetal Specialist to join Harry Farb, M.D. in the practice of Maternal Fetal Medicine. This position is best suited for an individual who likes personal, direct, hands-on patient care, teaching and outreach activities. Experience in CVS and PUBS is required. An excellent financial package, support services and personal and professional growth is offered by this opportunity.

Send CV to:

Harry Farb, M.D.
Medical Director
North Memorial Perinatal Center
3300 Oakdale North
Robbinsdale, Minnesota 55422
(612) 520-5950

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With a commitment to excellence and a focus on the total needs of each patient, Riverside Medical Center blends a 'primary' approach with major centers of excellence in cardiology, chemical dependency, mental health, neuro-sensory, obstetrics and orthopedics.

Picture yourself as a physican partner in the Minneapolis/St. Paul area's most comprehensive, integrated, primary care-based system of health care.

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Contact: **Natalie Odell**
Fairview Clinic Services
600 West 98th St, Suite 390
Bloomington, MN 55420
612-885-6224 1-800-842-6469

PERINATAL ASSOCIATES OF NORTHERN CALIFORNIA

Are you a clinical perinatologist? There is still time to join 4 perinatologists and one medical geneticist looking for one more perinatologist who: desires superior environment for patient care, in a private, modern level 3 facility doing 6,000 deliveries a year with superior perinatal outcome; likes personal, direct hands-on patient care; needs PUBS, IUTxF, CVS and complicated pregnancies, level 2 ultrasound; demands partners, not superiors; wants to live in clean, warm, not humid, snow-free (but 60 minutes from skiing) Sacramento, California; feels one's family has priorities; and is committed to the private practice of medicine. For more information, call (916) 733-1755.

Roger L. Wallace, D.O.
Perinatal Associates
of Northern California
5301 F. Street #110
Sacramento, California 95819

Beautiful Finger Lakes Region Ithaca, New York

Join comprehensive Ob-Gyn service affiliated with a successful Planned Parenthood agency. Unique opportunity for patient-oriented physician. Currently seeking a second BC/BE Ob-Gyn to provide out-patient gynecology, obstetrics, surgery, infertility, and abortions. Progressive university community (home of Cornell University) offers cultural attractions of a city with the benefits of small town living. Competitive salary, excellent benefits including malpractice. Available immediately.

Send CV To: Joyce Leslie, M.D., Planned Parenthood of Tompkins County, 314 West State Street, Ithaca, NY 14850

THE DEPARTMENT OF OBSTETRICS AND GYNECOLOGY UNIVERSITY OF WISCONSIN MEDICAL SCHOOL

is seeking applicants for two (2) additional full-time faculty positions in MATERNAL FETAL MEDICINE for a new women's health center and academic program at its Milwaukee Clinical Campus. The current faculty includes two perinatologists.

Sinai Samaritan Medical Center has recently consolidated its obstetrical and gynecological services in a redesigned facility in downtown Milwaukee. The department has the largest perinatal service in Wisconsin (5500 deliveries) and has a dedicated antenatal testing and perinatal ultrasonography unit, a midwifery, and adolescent pregnancy service. The department has an affiliation with St. Luke's Medical Center in Milwaukee for its residency program.

Qualifications include board candidacy or certification in maternal-fetal medicine and a commitment to teaching residents, medical students, and clinical research. Competitive salary package. Contact:

Chester B. Martin, M.D.
Chairman
Department of Obstetrics/Gynecology
University of Wisconsin Medical School
600 Highland Avenue, H4/654 CSC
Madison, WI 53792

Fredrik F. Broekhuizen, M.D.
Chairman and Chief
Milwaukee Campus
Department of Obstetrics/Gynecology
University of Wisconsin Medical School
Sinai Samaritan Medical Center
2000 West Kilbourn Avenue
Milwaukee, WI 53233
(414) 937-5600

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REPRODUCTIVE ENDOCRINOLOGIST

St. Luke's/Roosevelt Hospital Center is a major 1315-bed teaching hospital affiliated with Columbia University College of Physicians and Surgeons. Currently, our Department of Obstetrics and Gynecology seeks a Board Certified Subspecialist to direct the Division of Reproductive Endocrinology. The department has approved residency and medical student training programs.

We offer an attractive New York City location on the West Side of Manhattan. Salary, hospital and university appointments will be commensurate with qualifications and experience. Contact **David B. Crawford, Jr, MD, Acting Director, Obstetrics and Gynecology, St. Luke's/Roosevelt Hospital Center, Brodsky Building, Suite 5B, New York, NY 10019.** An Equal Opportunity Employer M/F.

ST. LUKE'S\ROOSEVELT
Hospital Center

OB/GYN Subspecialists

Gynecologic Oncology
Perinatology
Reproductive Endocrinology
Urogynecology

The University of South Dakota School of Medicine's Department of Obstetrics and Gynecology is building a full time academic team. We are located in Sioux Falls, South Dakota, a regional medical community of 120,000 serving South Dakota and three neighboring states.

Challenging positions are available for qualified candidates interested in pioneering medical education, research development and clinical practice.

A new comprehensive University-Health Science Center is being constructed which will house our program. Outreach and Indian Health opportunities are also available.

Financial package and incentives are competitive and commensurate with experience, credentials and rank.

Please send Curriculum Vitae to: R. H. Wiebe, M.D., Professor and Chairman, Department of Obstetrics and Gynecology, c/o Physician Placement Program, 1100 South Euclid Avenue, Sioux Falls, South Dakota 57117-5039 or call 800-468-3333 for more information.

ST. GEORGE'S HOSPITAL MEDICAL SCHOOL (University of London)

UNITED KINGDOM

SENIOR LECTURER/CONSULTANT OBSTETRICIAN AND GYNAECOLOGIST

A Senior Lecturer/Honorary Consultant is required for a busy South London Teaching Unit with a delivery rate of approximately 4000 a year. This is a partly clinical/academic/research post and an interest in perinatology or some specific research would be appreciated and facilities will be provided for appropriate research. St. Helier and St. George's Hospitals are linked University Hospitals. The majority of the clinical work will be done at St. Helier Hospital with two sessions at St. George's.

The salary will be in the range £35,390-£46,390 plus £1,292 London Allowance per annum in the scale for Senior Lecturers holding an Honorary Consultant contract. These salaries were subject to review from 1 April 1992.

For further information please contact Dr. D. Moncrieff at St. Helier Hospital, Wrythe Lane, Carshalton, Surrey, SM5 1AA, England (Telephone 081-644 4343, Fax 081-641 4546). Dr. Moncrieff will be visiting Toronto in June and would be happy to see interested applicants there.

Further details and an application form from the Personnel Officer, St. George's Medical School, Cranmer Terrace, London SW17 ORE, 081-784 2791 (24-hour answerphone). Closing date 3 July 1992. Please quote reference 35/92.

ASSISTANT/ASSOCIATE PROFESSOR MATERNAL-FETAL MEDICINE

The University of North Carolina at Chapel Hill Department of Obstetrics and Gynecology in conjunction with the Wake Medical Center, Raleigh, North Carolina, and the Wake Area Health Education Center has an opening for a board certified or active candidate perinatologist with a special interest in high risk pregnancies and ultrasound. The position is in the Wake AHEC OB/GYN Department which has three full-time obstetricians and gynecologists, plus the program director who is board certified in Maternal-Fetal Medicine. Wake Medical Center is located in Raleigh, approximately 40 minutes from Chapel Hill, and is a 560-bed comprehensive tertiary care center with over 4000 deliveries per year and a neonatal intensive care unit staffed by four neonatologists. Excellent opportunity for research and teaching residents and medical students rotating through Wake Medical Center at all times. Collaboration with the Maternal-Fetal Medicine Division at Chapel Hill is encouraged and anticipated. Competitive salary and excellent fringe benefits.

Write or contact:

Juan L. Granados, M.D.
OB/GYN Wake AHEC Director
Wake Medical Center
P.O. BOX 14465
Raleigh, NC 27620-4465
(919) 250-8535

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THE UNIVERSITY OF TEXAS SOUTHWESTERN MEDICAL CENTER AT DALLAS OBSTETRICS & GYNECOLOGY FACULTY POSITIONS

GENERALIST IN OB-GYN

Full and part-time positions available to qualified applicants who have completed an accredited residency in Ob-Gyn and are eligible for Texas licensure. Responsibilities will include the clinical care of women at Parkland Memorial Hospital and the Maternal Health and Family Planning Clinics as well as graduate and undergraduate teaching.

GYNECOLOGIC ONCOLOGY

Full time positions available to qualified applicants with special interest or expertise in molecular biology who have completed an accredited residency and Fellowship in Gynecologic Oncology and are eligible for Texas licensure. Responsibilites will include patient care including surgery and chemotherapy of gynecologic cancers; graduate and undergraduate teaching; research opportunities and participation in departmental continuing medical education efforts.

MATERNAL-FETAL MEDICINE/ REPRODUCTIVE ENDOCRINOLOGY

Full time positions available to qualified applicants who have completed an accredited residency and a 2-year Fellowship in either Maternal Fetal Medicine or Reproductive Endocrinology and are eligible for Texas licensure. Responsibilities willl include patient care; graduate and undergraduate teaching; research opportunities and participation in departmental continuing medical education efforts.

Salary and academic rank commensurate with curriculum vitae and experience.

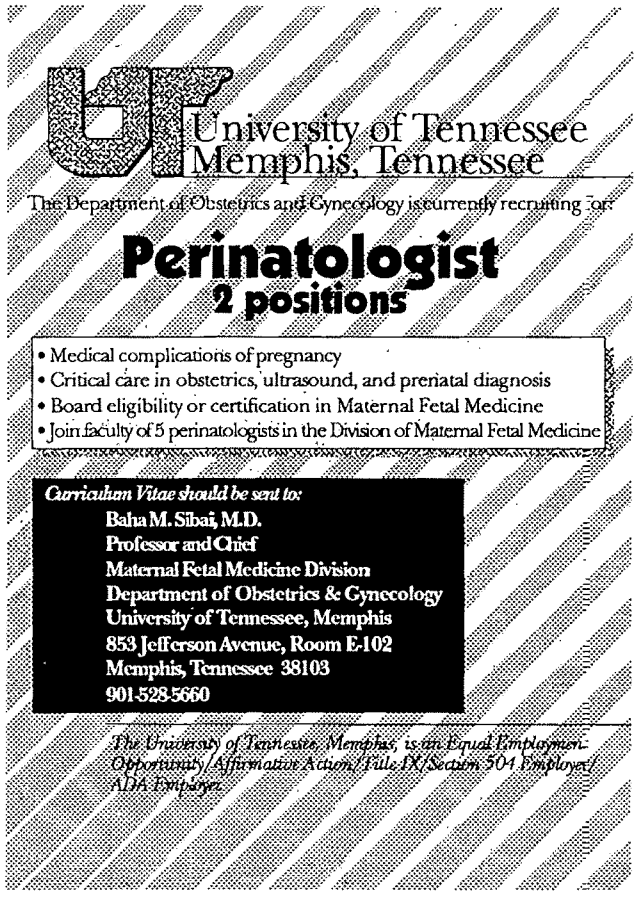
Interested persons send curriculum vitae to: F. Gary Cunningham, M.D., Professor and Chairman, Department Ob-Gyn, The University of Texas Southwestern Medical Center at Dallas, 5323 Harry Hines Boulevard, Dallas TX 75235-9032.

AN EQUAL OPPORTUNITY EMPLOYER

The Department of Obstetrics and Gynecology, University of Pennsylvania Medical Center, invites nominations and applications for the position of Profesor of Obstetrics and Gynecology in the School of Medicine and Director, Division of Gynecologic Oncology. Candidates must be board certified in Obstetrics and Gynecology and hold a certificate of special competence in Gynecologic Oncology from The American Board of Obstetrics and Gynecology, Inc. Candidates must be eligible for medical licensure in Pennsylvania. Responsibilities include the leadership and continued development of a division and fellowship program committed to excellence in teching, research and patient care. Demonstrated research accomplishments and excellent clinical skills are required. Salary is negotiable. Applications and nominations should include a current curriculum vitae and names and addresses of three references. They should be submitted to:

Michael T. Mennuti, M.D.
Professor and Chairman
Department of Obstetrics and Gynecology
Hospital of the University of Pennsylvania
3400 Spruce Street, 4 Gibson Building
Philadelphia, PA 19104-4283
Telephone: (215) 662-3234

The University of Pennsylvania is an equal opportunity/ affirmative action employer.



THE DEPARTMENT OF OBSTETRICS AND GYNECOLOGY UNIVERSITY OF WISCONSIN MEDICAL SCHOOL

is seeking applicants for two (2) full-time faculty positions for a new women's health center and academic program at its Milwaukee Clinical Campus.

Sinai Samaritan Medical Center has recently consolidated its obstetrical and gynecological services in a redesigned facility in downtown Milwaukee. The department supports a residency program, the largest perinatal service in Wisconsin (5500 deliveries), consultative ultrasound, genetic and infectious disease programs, and reproductive/endocrine services (including an IVF program). The department is affiliated with Planned Parenthood of Wisconsin and St. Luke's Medical Center in Milwaukee.

Qualifications include a board candidacy or certification in obstetrics and gynecology and a commitment to teaching residents, medical students, and clinical research. Qualifications for the two positions for GENERAL OB/GYN can take into consideration special individual interests. Special expertise of interest for the department are:

—STATEWIDE FAMILY PLANNING
(Part-time medical director, Planned Parenthood)
—OPERATIVE LAPAROSCOPY, HYSTEROSCOPY
—UROGYNECOLOGY
—OB/GYN ULTRASOUND

Private practice opportunities are also available in an associated offsite multispecialty clinic and all faculty members participate in a group practice at the institution. Contact:

Chester B. Martin, M.D.
Chairman
Department of Obstetrics and Gynecology
University of Wisconsin Medical Schl.
600 Highland Avenue,
H4/654 CSC
Madison, WI 53792

Fredrik F. Broekhuizen, M.D.
Chairman and Chief
Milwaukee Campus
Department of Obstetrics/Gynecology
University of Wisconsin Medical Schl.
Sinai Samaritan Medical Center
2000 West Kilbourn Avenue
Milwaukee, WI 53233
(414) 937-5600

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Maternal-Fetal Medicine Faculty
Department of Obstetrics and Gynecology
University of Medicine and Dentistry of New Jersey
Robert Wood Johnson Medical School
Three Positions Available

The Department of Obstetrics and Gynecology of the UMDNJ-Robert Wood Johnson Medical School invites applications for three full-time faculty positions as Specialists in Maternal-Fetal Medicine. Candidates should be board certified or eligible in Maternal Fetal Medicine. The positions involve academic practice, graduate and undergraduate medical education, and clinical and/or laboratory research.

The Division is located at St. Peter's Medical Center which has the largest obstetrical and neonatal program in the State. MFM Fellowship approved. Four MFM specialists, four sonographers, a geneticist, two research nurses and one epidemiologist/biostatistician are currently in the Division. Academic Rank and competitive salary commensurate with level of experience and qualification.

Send inquiries and curriculum vitae to:

Robert A. Knuppel, M.D., M.P.H.
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IMPORTANT NOTE—This information is a BRIEF SUMMARY of the complete prescribing information provided with the product and therefore should not be used as the basis for prescribing the product. This summary was prepared by deleting from the complete prescribing information certain text, tables and references. The physician should be thoroughly familiar with the complete prescribing information before prescribing the product.

INDICATIONS AND USAGE: PREVENTION OF PREGNANCY.

CONTRAINDICATIONS: Oral contraceptives should not be used in women who currently have the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophlebitis or thromboembolic disorders. 3. Cerebral vascular or coronary artery disease. 4. Known or suspected carcinoma of the breast. 5. Carcinoma of the endometrium or other known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Cholestatic jaundice of pregnancy or jaundice with prior pill use. 8. Hepatic adenomas or carcinomas. 9. Known or suspected pregnancy.

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risks of several serious conditions including myocardial infarction, thromboembolism, stroke, hepatic neoplasia, and gallbladder disease, although the risk of serious morbidity or mortality is very small in healthy women without underlying risk factors. The risk of morbidity and mortality increases significantly in the presence of other underlying risk factors such as hypertension, hyperlipidemias, obesity and diabetes. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks. The information contained in this brief summary is principally based on studies carried out in patients who used oral contraceptives with higher formulations of estrogens and progestogens than those in common use today. The effect of long term use of the oral contraceptives with lower formulations of both estrogens and progestogens remains to be determined. Throughout this brief summary epidemiological studies reported are of two types: retrospective or case control studies and prospective or cohort studies. Case control studies provide a measure of the relative risk of a disease, namely a *ratio* of the incidence of a disease among oral contraceptive users to that among nonusers. The relative risk does not provide information on the actual clinical occurrence of a disease. Cohort studies provide a measure of attributable risk, which is the *difference* in the incidence of disease between oral contraceptive users and nonusers. The attributable risk does provide information about the actual occurrence of a disease in the population. For further information, the reader is referred to a text on epidemiological methods. 1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS. a. Myocardial Infarction. An increased risk of myocardial infarction has been associated with oral contraceptive use. This risk is primarily in smokers or women with other underlying risk factors for coronary artery disease such as hypertension, hypercholesterolemia, morbid obesity, and diabetes. The relative risk of heart attack for current oral contraceptive users has been estimated to be two to six. The risk is very low under the age of 30. Smoking in combination with oral contraceptive use has been shown to contribute substantially to the incidence of myocardial infarctions in women in their mid-thirties or older with smoking accounting for the majority of excess cases. Mortality rates associated with circulatory disease have been shown to increase substantially in smokers, especially in those 35 years of age and older among women who use oral contraceptives. Oral contraceptives may compound the effects of well-known risk factors, such as hypertension, diabetes, hyperlipidemias, age and obesity. In particular, some progestogens are known to decrease HDL cholesterol and cause glucose intolerance, while estrogens may create a state of hyperinsulinism. Oral contraceptives have been shown to increase blood pressure among users (see section 9 in WARNINGS). Similar effects on risk factors have been associated with an increased risk of heart disease. Oral contraceptives must be used with caution in women with cardiovascular disease risk factors. b. Thromboembolism. An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Case control studies have found the relative risk of users compared to non-users to be 3 for the first episode of superficial venous thrombosis, 4 to 11 for deep vein thrombosis or pulmonary embolism, and 1.5 to 6 for women with predisposing conditions for venous thromboembolic disease. Cohort studies have shown the relative risk to be somewhat lower, about 3 for new cases and about 4.5 for new cases requiring hospitalization. The risk of thromboembolic disease associated with oral contraceptives is not related to length of use and disappears after pill use is stopped. A two- to four-fold increase in relative risk of post-operative thromboembolic complications has been reported with the use of oral contraceptives. The relative risk of venous thrombosis in women who have predisposing conditions is twice that of women without such medical conditions. If feasible, oral contraceptives should be discontinued at least four weeks prior to and for two weeks after elective surgery of a type associated with an increase in risk of thromboembolism and during and following prolonged immobilization. Since the immediate postpartum period is also associated with an increased risk of thromboembolism, oral contraceptives should be started no earlier than four weeks after delivery in women who elect not to breast feed. c. Cerebrovascular diseases. Oral contraceptives have been shown to increase both the relative and attributable risks of cerebrovascular events (thrombotic and hemorrhagic strokes), although, in general, the risk is greatest among older (>35 years), hypertensive women who also smoke. Hypertension was found to be a risk factor for both users and non-users, for both types of strokes, and smoking interacted to increase the risk of stroke. In a large study, the relative risk of thrombotic strokes has been shown to range from 3 for normotensive users to 14 for users with severe hypertension. The relative risk of hemorrhagic stroke is reported to be 1.2 for nonsmokers who used oral contraceptives, 2.6 for smokers who did not use oral contraceptives, 7.6 for smokers who used oral contraceptives, 1.8 for normotensive users and 25.7 for users with severe hypertension. The attributable risk is also greater in older women. d. Dose-related risk of vascular disease from oral contraceptives. A positive association has been observed between the amount of estrogen and progestogen in oral contraceptives and the risk of vascular disease. A decline in serum high density lipoproteins (HDL) has been reported with many progestational agents. A decline in serum high density lipoproteins has been associated with an increased incidence of ischemic heart disease. Because estrogens increase HDL cholesterol, the net effect of an oral contraceptive depends on a balance achieved between doses of estrogen and progestogen and the activity of the progestogen used in the contraceptive. The activity and amount of both hormones should be considered in the choice of an oral contraceptive. Minimizing exposure to estrogen and progestogen is in keeping with good principles of therapeutics. For any particular estrogen/progestogen combination, the dosage regimen prescribed should be one which contains the least amount of estrogen and progestogen that is compatible with a low failure rate and the needs of the individual patient. New acceptors of oral contraceptive agents should be started on preparations containing 0.035 mg or less of estrogen. e. Persistence of risk of vascular disease. There are two studies which have shown persistence of risk of vascular disease for ever-users of oral contraceptives. In a study in the United States, the risk of developing myocardial infarction after discontinuing oral contraceptives persists for at least 9 years for women 40-49 years who had used oral contraceptives for five or more years, but this increased risk was not demonstrated in other age groups. In another study in Great Britain, the risk of developing cerebrovascular disease persisted for at least 6 years after discontinuation of oral contraceptives, although excess risk was very small. However, both studies were performed with oral contraceptive formulations containing 50 micrograms or higher of estrogens. 2. ESTIMATES OF MORTALITY FROM CONTRACEPTIVE USE. One study gathered data from a variety of sources which have estimated the mortality rate associated with different methods of contraception at different ages. These estimates include the combined risk of death associated with contraceptive methods plus the risk attributable to pregnancy in the event of method failure. Each method of contraception has its specific benefits and risks. The study concluded that with the exception of oral contraceptive users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of birth control is low and below that associated with childbirth. However, smokers 35 and older and non-smokers 40 and older who use oral contraceptives have a significant increase in mortality higher than those using other methods of birth control. These facts must be weighed in conjunction with failure rates for other methods and the risk associated with subsequent pregnancy. 3. CARCINOMA OF THE REPRODUCTIVE ORGANS. Numerous epidemiological studies have been performed on the incidence of breast, endometrial, ovarian and cervical cancer in women using oral contraceptives. While there are conflicting reports most studies suggest that use of oral contraceptives is not associated with an overall increase in the risk of developing breast cancer. Some studies have reported an increased relative risk of developing breast cancer, particularly at a younger age. This increased relative risk appears to be related to duration of use. Some studies suggest that oral contraceptive use has been associated with an increase in the risk of cervical intraepithelial neoplasia in some populations of women. However, there continues to be controversy about the extent to which such findings may be due to differences in sexual behavior and other factors. 4. HEPATIC NEOPLASIA. Benign hepatic adenomas are associated with oral contraceptive use, although the incidence of benign tumors is rare in the United States. Indirect calculations have estimated the attributable risk to be in the range of 3.3 cases/100,000 for users, a risk that increases after four or more years of use especially with oral contraceptives of higher dose. Rupture of benign, hepatic adenomas may cause death through intra-abdominal hemorrhage. Studies from Britain have shown an increased risk of developing hepatocellular carcinoma in long-term (>8 years) oral contraceptive users. However, these cancers are rare in the U.S. and the attributable risk (the excess incidence) of liver cancers in oral contraceptive users approaches less than one per million users. 5. OCULAR LESIONS. There have been clinical case reports of retinal thrombosis associated with the use of oral contraceptives. Oral contraceptives should be discontinued if there is unexplained partial or complete loss of vision; onset of proptosis or diplopia, papilledema, or retinal vascular lesions. Appropriate diagnostic and therapeutic measures should be undertaken immediately. 6. ORAL CONTRACEPTIVE USE BEFORE OR DURING EARLY PREGNANCY. Extensive epidemiological studies have revealed no increased risk of birth defects in women who have used oral contraceptives prior to pregnancy. The majority of recent studies also do not indicate a teratogenic effect, particularly in so far as cardiac anomalies and limb reduction defects are concerned, when taken inadvertently during early pregnancy. The administration of oral contraceptives to induce withdrawal bleeding should not be used as a test for pregnancy. Oral contraceptives should not be used during pregnancy to treat threatened or habitual abortion. It is recommended that for any patient who has missed two consecutive periods pregnancy should be ruled out before continuing oral contraceptive use. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period. Oral contraceptive use should be discontinued until pregnancy is ruled out. 7. GALLBLADDER DISEASE. Earlier studies have reported an increased lifetime relative risk of gallbladder surgery in users of oral contraceptives and estrogens. More recent studies, however, have shown that the relative risk of developing gallbladder disease among oral contraceptive users may be minimal. The recent findings of minimal risk may be related to the use of oral contraceptive formulations containing lower hormonal doses of estrogens and progestogens. 8. CARBOHYDRATE AND LIPID METABOLIC EFFECTS. Oral contraceptives have been shown to cause a decrease in glucose tolerance in a significant percentage of users. This effect has been shown to be directly related to estrogen dose. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents. However, in the non-diabetic woman, oral contraceptives appear to have no effect on fasting blood glucose. Because of these demonstrated effects prediabetic and diabetic women in particular should be carefully monitored while taking oral contraceptives. A small proportion of women will have persistent hypertriglyceridemia while on the pill. As discussed earlier (see WARNINGS 1a and 1d), changes in serum triglycerides and lipoprotein levels have been reported in oral contraceptive users. 9. ELEVATED BLOOD PRESSURE. An increase in blood pressure has been reported in women taking oral contraceptives, and this increase is more likely in older oral contraceptive users and with extended duration of use. Data from the Royal College of General Practitioners and subsequent randomized trials have shown that the incidence of hypertension increases with increasing progestational activity. Women with a history of hypertension or hypertension-related diseases, or renal disease, should be encouraged to use another method of contraception. If women elect to use oral contraceptives, they should be monitored closely and if significant elevation of blood pressure occurs, oral contraceptives should be discontinued. For most women, elevated blood pressure will return to normal after stopping oral contraceptives, and there is no difference in the occurrence of hypertension between former and never users. 10. HEADACHE. The onset or exacerbation of migraine or development of headache with a new pattern which is recurrent, persistent or severe requires discontinuation of oral contraceptives and evaluation of the cause. 11. BLEEDING IRREGULARITIES. Breakthrough bleeding and spotting are sometimes encountered in patients on oral contraceptives, especially during the first three months of use. Non-hormonal causes should be considered and adequate diagnostic measures taken to rule out malignancy or pregnancy in the event of breakthrough bleeding as in the case of any abnormal vaginal bleeding. If pathology has been excluded, time or a change to another formulation may solve the problem. In the event of amenorrhea, pregnancy should be ruled out. Some women may encounter post-pill amenorrhea or oligomenorrhea, especially when such a condition was preexistent. 12. ECTOPIC PREGNANCY. Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. **PRECAUTIONS:** 1. PHYSICAL EXAMINATION AND FOLLOW UP. A complete medical history and physical examination should be taken prior to the initiation or reinstitution of oral contraceptives and at least annually during use of oral contraceptives. These physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including cervical cytology, and relevant laboratory tests. In case of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be conducted to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care. 2. LIPID DISORDERS. Women who are being treated for hyperlipidemias should be followed closely if they elect to use oral contraceptives. Some progestogens may elevate LDL levels and may render the control of hyperlipidemias more difficult. 3. LIVER FUNCTION. If jaundice develops in any woman receiving such drugs, the medication should be discontinued. Steroid hormones may be poorly metabolized in patients with impaired liver function. 4. FLUID RETENTION. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention. 5. EMOTIONAL DISORDERS. Women with a history of depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. 6. CONTACT LENSES. Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist. 7. DRUG INTERACTIONS. Reduced efficacy and increased incidence of breakthrough bleeding and menstrual irregularities have been associated with concomitant use of rifampin. A similar association, though less marked, has been suggested with barbiturates, phenylbutazone, phenytoin sodium, and possibly with griseofulvin, ampicillin and tetracyclines. 8. INTERACTIONS WITH LABORATORY TESTS. Certain endocrine and liver function tests and blood components may be affected by oral contraceptives: a. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3, increased norepinephrine-induced platelet aggregability. b. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or by radio-immunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. c. Other binding proteins may be elevated in serum. d. Sex-binding globulins are increased and result in elevated levels of total circulating sex steroids and corticoids; however, free or biologically active levels remain unchanged. e. Triglycerides may be increased. f. Glucose tolerance may be decreased. g. Serum folate levels may be depressed by oral contraceptive therapy. This may be of clinical significance if a woman becomes pregnant shortly after discontinuing oral contraceptives. 9. CARCINOGENESIS. See WARNINGS section. 10. PREGNANCY. Pregnancy Category X. See CONTRAINDICATIONS and WARNINGS sections. 11. NURSING MOTHERS. Small amounts of oral contraceptive steroids have been identified in the milk of nursing mothers and a few adverse effects on the child have been reported, including jaundice and breast enlargement. In addition, oral contraceptives given in the postpartum period may interfere with lactation by decreasing the quantity and quality of breast milk. If possible, the nursing mother should be advised not to use oral contraceptives but to use other forms of contraception until she has completely weaned her child. **INFORMATION FOR THE PATIENT: See Patient Package Insert.**

ADVERSE REACTIONS: An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS section). Thrombophlebitis and venous thrombosis with or without embolism. Arterial thromboembolism. Pulmonary embolism. Myocardial infarction. Cerebral hemorrhage. Cerebral thrombosis. Hypertension. Gallbladder disease. Hepatic adenomas or benign liver tumors. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea. Vomiting. Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding. Spotting. Change in menstrual flow. Amenorrhea. Temporary infertility after discontinuation of treatment. Edema. Melasma which may persist. Breast changes: tenderness, enlargement, secretion. Change in weight (increase or decrease). Change in cervical erosion and secretion. Diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives and the association has been neither confirmed nor refuted: Pre-menstrual syndrome. Cataracts. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiform. Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. Hemolytic uremic syndrome. Acne. Changes in libido. Colitis.

OVERDOSAGE: Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea and withdrawal bleeding may occur in females.

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Please see complete Prescribing Information, a brief summary of which is on the preceding page.

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1. Droegemueller W, Adamson DG, Brown D, et al. Three-day treatment with butoconazole nitrate for vulvovaginal candidiasis. *Obstet Gynecol.* 1984;64:530-534. *Micrographs on file, Syntex Laboratories, Inc. †In pregnant patients (second and third trimesters only), therapy should be extended to 6 days. Please see adjacent page for brief summary of prescribing information. © 1991 Syntex Laboratories, Inc. 813-12-196-91

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CONTRAINDICATIONS: FEMSTAT is contraindicated in patients hypersensitive to any of the ingredients.

PRECAUTIONS: General: If clinical symptoms persist, repeat microbiological tests to rule out other pathogens and confirm the diagnosis. Discontinue drug if sensitization or irritation occurs.

Information for the Patient: Do not discontinue prematurely during menstruation or because of symptomatic relief.

Carcinogenesis: Animal studies have not been done.

Mutagenesis: Mutagenicity studies were negative.

Impairment of Fertility: Animal studies showed no impairment of fertility.

Pregnancy Category C: Adverse effects were noted in animals treated with high oral doses. No studies were done in women during first trimester. Patients in the second or third trimester have shown no adverse effects attributable to the drug.

Nursing Mothers: Use with caution.

Pediatric Use: Safety and efficacy have not been established.

ADVERSE REACTIONS: Vulvar/vaginal burning in 2.3% of patients, vulvar itching in 0.9%, discharge, soreness, swelling, itching of fingers each in 0.2%. Complaints caused 1.6% to discontinue drug.

DOSAGE AND ADMINISTRATION: Non-pregnant Patients: The recommended dose is one applicatorful of cream (approximately 5 grams) intravaginally at bedtime for three days. Treatment can be extended for an additional three days if necessary.

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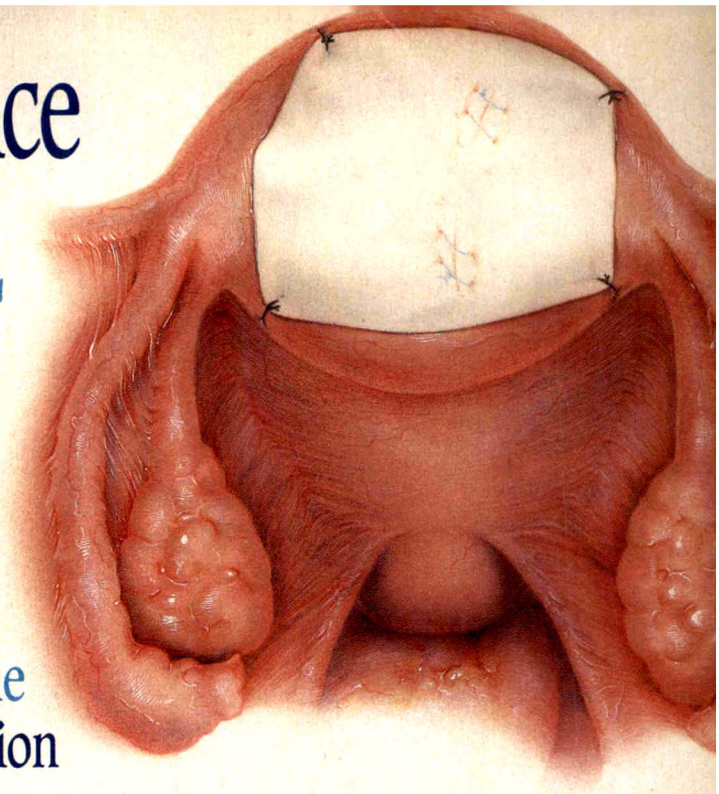
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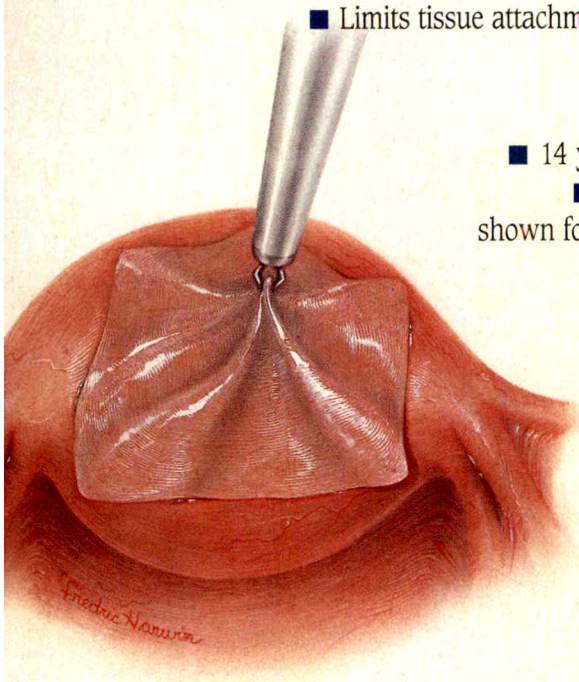


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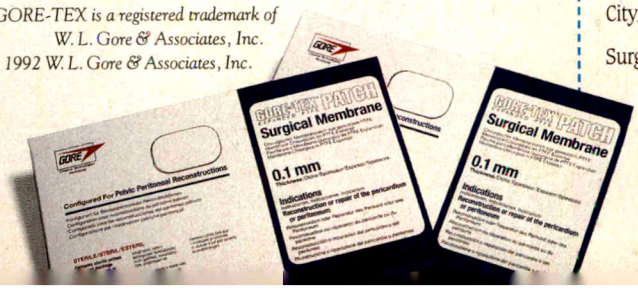
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Part 1
1992

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Vol. 166, No. 6, Part 1, June 1992. The American Journal of Obstetrics and Gynecology (ISSN 0002-9378) is published monthly, except semimonthly in January (thirteen issues per year), by Mosby–Year Book, Inc., 11830 Westline Industrial Drive, St. Louis, MO 63146-3318. Second-class postage is paid at St. Louis, Missouri, and additional mailing offices. POSTMASTER: Send address changes to American Journal of Obstetrics and Gynecology, Mosby–Year Book, Inc., 11830 Westline Industrial Drive, St. Louis, MO 63146-3318, (800) 325-4177, ext. 4351, or (314) 453-4351. Annual subscription rates for 1992: domestic, $96.00 for individuals and $169.00 for institutions. Printed in the U.S.A. Copyright © 1992 by Mosby–Year Book, Inc. All rights reserved. No part of this publication may be reproduced, stored, or transmitted in any form or by any means, electronic or mechanical, including photocopy, recording, or any information storage and retrieval system, without permission in writing from the Publisher.

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San Francisco, California, Birmingham, Alabama, Columbus, Ohio, and Houston, Texas

The incidence of major morbidities in 20,680 singleton live inborn neonates is presented by gestational age and birth weight.

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INDICATIONS AND USAGE
TREATMENT

CEFOTAN is indicated for the therapeutic treatment of the following infections when caused by susceptible strains of the designated organisms:

Urinary Tract Infections caused by *E. coli, Klebsiella* species (including *K. pneumoniae*), *Proteus mirabilis,* and *Proteus* sp (which may include the organisms now called *Proteus vulgaris, Providencia rettgeri,* and *Morganella morganii*).

Lower Respiratory Tract Infections caused by *Streptococcus pneumoniae* (formerly *D. pneumoniae*), *Staphylococcus aureus* (penicillinase- and nonpenicillinase-producing strains), *Haemophilus influenzae* (including ampicillin-resistant strains), *Klebsiella* species (including *K. pneumoniae*), *E. coli, Proteus mirabilis,* and *Serratia marcescens**.

Skin and Skin Structure Infections due to *Staphylococcus aureus* (penicillinase- and nonpenicillinase-producing strains), *Staphylococcus epidermidis, Streptococcus pyogenes, Streptococcus* species (excluding enterococci), *Escherichia coli, Klebsiella pneumoniae, Peptococcus niger*, Peptostreptococcus* species.

Gynecologic Infections caused by *Staphylococcus aureus* (including penicillinase- and nonpenicillinase-producing strains), *Staphylococcus epidermidis, Streptococcus* species (excluding enterococci), *Streptococcus agalactiae, E. coli, Proteus mirabilis, Neisseria gonorrhoeae,* Bacteroides species (excluding *B. distasonis, B. ovatus, B. thetaiotaomicron*), *Fusobacterium* species*, and gram-positive anaerobic cocci (including *Peptococcus niger* and *Peptostreptococcus* species).

Cefotetan, like other cephalosporins, has no activity against *Chlamydia trachomatis.* Therefore, when cephalosporins are used in the treatment of pelvic inflammatory disease, and *C. trachomatis* is one of the suspected pathogens, appropriate antichlamydial coverage should be added.

Intra-abdominal Infections caused by *E. coli, Klebsiella* species (including *K. pneumoniae*), *Streptococcus* species (excluding enterococci), *Bacteroides* species (excluding *B. distasonis, B. ovatus, B. thetaiotaomicron*) and *Clostridium* species*.

Bone and Joint Infections caused by *Staphylococcus aureus**.

*Efficacy for this organism in this organ system was studied in fewer than ten infections.

Specimens for bacteriological examination should be obtained in order to isolate and identify causative organisms and to determine their susceptibilities to cefotetan. Therapy may be instituted before results of susceptibility studies are known; however, once these results become available, the antibiotic treatment should be adjusted accordingly.

In cases of confirmed or suspected gram-positive or gram-negative sepsis or in patients with other serious infections in which the causative organism has not been identified, it is possible to use CEFOTAN concomitantly with an aminoglycoside. Cefotetan combinations with aminoglycosides have been shown to be synergistic *in vitro* against many Enterobacteriaceae and also some other gram-negative bacteria. The dosage recommended in the labeling of both antibiotics may be given and depends on the severity of the infection and the patient's condition.

NOTE: If CEFOTAN and an aminoglycoside are used concomitantly, renal function should be carefully monitored, especially if higher dosages of the aminoglycoside are to be administered or if therapy is prolonged, because of the potential nephrotoxicity and ototoxicity of aminoglycosidic antibiotics. Although, to date, nephrotoxicity has not been noted when CEFOTAN was given alone, it is possible that nephrotoxicity may be potentiated if CEFOTAN is used concomitantly with an aminoglycoside.

PROPHYLAXIS

The preoperative administration of CEFOTAN may reduce the incidence of certain postoperative infections in patients undergoing surgical procedures that are classified as clean contaminated or potentially contaminated (e.g., cesarean section, abdominal or vaginal hysterectomy, transurethral surgery, biliary tract surgery, and gastrointestinal surgery).

The prophylactic dose of CEFOTAN should be administered 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, CEFOTAN should be administered intravenously after the clamping of the umbilical cord.

If there are signs and symptoms of infection, specimens for culture should be obtained for identification of the causative organism so that appropriate therapeutic measures may be initiated.

CONTRAINDICATIONS

CEFOTAN is contraindicated in patients with known allergy to the cephalosporin group of antibiotics.

WARNINGS

BEFORE THERAPY WITH CEFOTAN IS INSTITUTED, CAREFUL INQUIRY SHOULD BE MADE TO DETERMINE WHETHER THE PATIENT HAS HAD PREVIOUS HYPERSENSITIVITY REACTIONS TO CEFOTETAN DISODIUM, CEPHALOSPORINS, PENICILLINS, OR OTHER DRUGS. IF THIS PRODUCT IS TO BE GIVEN TO PENICILLIN-SENSITIVE PATIENTS, CAUTION SHOULD BE EXERCISED BECAUSE CROSS-HYPERSENSITIVITY AMONG BETA-LACTAM ANTIBIOTICS HAS BEEN CLEARLY DOCUMENTED AND MAY OCCUR IN UP TO 10% OF PATIENTS WITH A HISTORY OF PENICILLIN ALLERGY. IF AN ALLERGIC REACTION TO CEFOTAN OCCURS, DISCONTINUE THE DRUG. SERIOUS ACUTE HYPERSENSITIVITY REACTIONS MAY REQUIRE TREATMENT WITH EPINEPHRINE AND OTHER EMERGENCY MEASURES, INCLUDING OXYGEN, INTRAVENOUS FLUIDS, INTRAVENOUS ANTIHISTAMINES, CORTICOSTEROIDS, PRESSOR AMINES AND AIRWAY MANAGEMENT, AS CLINICALLY INDICATED.

Pseudomembranous colitis has been reported with nearly all antibacterial agents, including cefotetan, and may range from mild to life-threatening. Onset of pseudomembranous colitis symptoms may occur during or after antibiotic treatment or surgical prophylaxis. Therefore, it is important to consider this diagnosis in patients who present with diarrhea subsequent to the administration of antibacterial agents.

Treatment with antibacterial agents alters the normal flora of the colon and may permit overgrowth of clostridia. Studies indicate that a toxin produced by *Clostridium difficile* is a primary cause of "antibiotic-associated colitis."

After the diagnosis of pseudomembranous colitis has been established, therapeutic measures should be initiated. Mild cases of pseudomembranous colitis usually respond to discontinuation of the drug alone. In moderate to severe cases, consideration should be given to management with fluids and electrolytes, protein supplementation, and treatment with an oral antibacterial drug effective against *Clostridium difficile.*

In common with many other broad-spectrum antibiotics, CEFOTAN may be associated with a fall in prothrombin activity and, possibly, subsequent bleeding. Those at increased risk include patients with renal or hepatobiliary impairment or poor nutritional state, the elderly, and patients with cancer. Prothrombin time should be monitored and exogenous vitamin K administered as indicated.

PRECAUTIONS

General: As with other broad-spectrum antibiotics, prolonged use of CEFOTAN may result in overgrowth of nonsusceptible organisms. Careful observation of the patient is essential. If superinfection does occur during therapy, appropriate measures should be taken.

CEFOTAN should be used with caution in individuals with a history of gastrointestinal disease, particularly colitis.

Information for Patients: As with some other cephalosporins, a disulfiram-like reaction characterized by flushing, sweating, headache, and tachycardia may occur when alcohol (beer, wine, etc.) is ingested within 72 hours after CEFOTAN administration. Patients should be cautioned about the ingestion of alcoholic beverages following the administration of CEFOTAN.

Drug Interactions: Although to date nephrotoxicity has not been noted when CEFOTAN was given alone, it is possible that nephrotoxicity may be potentiated if CEFOTAN is used concomitantly with an aminoglycoside.

Drug/Laboratory Test Interactions: The administration of CEFOTAN may result in a false-positive reaction for glucose in the urine using Clinitest®†, Benedict's solution, or Fehling's solution. It is recommended that glucose tests based on enzymatic glucose oxidase be used.

As with other cephalosporins, high concentrations of cefotetan may interfere with measurement of serum and urine creatinine levels by Jaffe reaction and produce false increases in the levels of creatinine reported.

Carcinogenesis, Mutagenesis, Impairment of Fertility: Although long-term studies in animals have not been performed to evaluate carcinogenic potential, no mutagenic potential of cefotetan was found in standard laboratory tests.

Cefotetan has adverse effects on the testes of prepubertal rats. Subcutaneous administration of 500 mg/kg/day (approximately 8-16 times the usual adult human dose) on days 6-35 of life (thought to be developmentally analogous to late childhood and prepuberty in humans) resulted in reduced testicular weight and seminiferous tubule degeneration in 10 of 10 animals. Affected cells included spermatogonia and spermatocytes; Sertoli and Leydig cells were unaffected. Incidence and severity of lesions were dose-dependent; at 120 mg/kg/day (approximately 2-4 times the usual human dose) only 1 of 10 treated animals was affected, and the degree of degeneration was mild.

Similar lesions have been observed in experiments of comparable design with other methylthiotetrazole-containing antibiotics and impaired fertility has been reported, particularly at high dose levels. No testicular effects were observed in 7-week-old rats treated with up to 1000 mg/kg/day SC for 5 weeks, or in infant dogs (3 weeks old) that received up to 300 mg/kg/day IV for 5 weeks. The relevance of these findings to humans is unknown.

Pregnancy: Teratogenic Effects. Pregnancy Category B: Reproduction studies have been performed in rats and monkeys at doses up to 20 times the human dose and have revealed no evidence of impaired fertility or harm to the fetus due to cefotetan. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproductive studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Nursing Mothers: Cefotetan is excreted in human milk in very low concentrations. Caution should be exercised when cefotetan is administered to a nursing woman.

Pediatric Use: Safety and effectiveness in children have not been established.

ADVERSE REACTIONS

In clinical studies, the following adverse effects were considered related to CEFOTAN therapy. Those appearing in italics have been reported during postmarketing experience.

Gastrointestinal symptoms occurred in 1.5% of patients; the most frequent were diarrhea (1 in 80) and nausea (1 in 700); *pseudomembranous colitis.* Onset of pseudomembranous colitis symptoms may occur during or after antibiotic treatment or surgical prophylaxis. (See WARNINGS.)

Hematologic laboratory abnormalities occurred in 1.4% of patients and included eosinophilia (1 in 200), positive direct Coombs' test (1 in 250), and thrombocytosis (1 in 300); *agranulocytosis, hemolytic anemia, leukopenia, thrombocytopenia,* and *prolonged prothrombin time.*

Hepatic enzyme elevations occurred in 1.2% of patients and included a rise in SGPT (1 in 150), SGOT (1 in 300), alkaline phosphatase (1 in 700), and LDH (1 in 700).

Hypersensitivity reactions were reported in 1.2% of patients and included rash (1 in 150) and itching (1 in 700); *anaphylactic reactions.*

Local effects were reported in less than 1.0% of patients and included phlebitis at the site of injection (1 in 300), and discomfort (1 in 500).

Miscellaneous: *Fever*

In addition to the adverse reactions listed above which have been observed in patients treated with cefotetan, the following adverse reactions and altered laboratory tests have been reported for cephalosporin-class antibiotics: urticaria, pruritis, Stevens-Johnson syndrome, erythema multiforme, toxic epidermal necrolysis, vomiting, abdominal pain, colitis, superinfection, vaginitis including vaginal candidiasis, renal dysfunction, toxic nephropathy, hepatic dysfunction including cholestasis, aplastic anemia, hemorrhage, increased BUN, increased creatinine, elevated bilirubin, pancytopenia, and neutropenia.

Several cephalosporins have been implicated in triggering seizures, particularly in patients with renal impairment when the dosage was not reduced (see DOSAGE AND ADMINISTRATION and OVERDOSAGE). If seizures associated with drug therapy occur, the drug should be discontinued. Anticonvulsant therapy can be given if clinically indicated.

DOSAGE AND ADMINISTRATION
TREATMENT

The usual adult dosage is 1 or 2 grams of CEFOTAN administered intravenously or intramuscularly every 12 hours for 5 to 10 days. Proper dosage and route of administration should be determined by the condition of the patient, severity of the infection, and susceptibility of the causative organism.

GENERAL GUIDELINES FOR DOSAGE OF CEFOTAN

Type of Infection	Daily Dose	Frequency and Route
Urinary Tract	1-4 grams	500 mg every 12 hours IV or IM 1 or 2 g every 24 hours IV or IM 1 or 2 g every 12 hours IV or IM
Skin & Skin Structure		
Mild-Moderate[a]	2 grams	2 g every 24 hours IV 1 g every 12 hours IV or IM
Severe	4 grams	2 g every 12 hours IV
Other Sites	2-4 grams	1 or 2 g every 12 hours IV or IM
Severe	4 grams	2 g every 12 hours IV
Life-Threatening	6 grams[b]	3 g every 12 hours IV

[a] *K. pneumoniae* skin and skin structure infections should be treated with 1 or 2 grams every 12 hours IV or IM.

[b] Maximum daily dosage should not exceed 6 grams.

If *C. trachomatis* is a suspected pathogen in gynecologic infections, appropriate antichlamydial coverage should be added, since cefotetan has no activity against this organism.

PROPHYLAXIS

To prevent postoperative infection in clean contaminated or potentially contaminated surgery in adults, the recommended dosage is 1 or 2 g of CEFOTAN administered once, intravenously, 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, the dose should be administered as soon as the umbilical cord is clamped.

IMPAIRED RENAL FUNCTION

When renal function is impaired, a reduced dosage schedule must be employed. See FULL PRESCRIBING INFORMATION for a complete description.

NOTE: Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration whenever solution and container permit.

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References: 1. Walker CK, Landers DV, Ohm-Smith MJ, et al. Comparison of cefotetan plus doxycycline with cefoxitin plus doxycycline in the inpatient treatment of acute salpingitis. *Sex Trans Dis.* 1991;18:119-123. **2.** McGregor JA, Gordon SF, Krotec J, Poindexter AN. Results of a randomized, multicenter, comparative trial of a single dose of cefotetan versus multiple doses of cefoxitin as prophylaxis in cesarean section. *Am J Obstet Gynecol.* 1988;158:701-706. **3.** Carver P, Quintiliani R, Nightingale CH. Comparative pharmacokinetic study of cefotetan and cefoxitin in healthy volunteers. *Infect Surg.* April 1986(suppl):11-14. **4.** Ohm-Smith MJ, Sweet RL, Hadley WK. In vitro activity of cefbuperazone and other antimicrobial agents against isolates from the female genital tract. *Antimicrob Agents Chemother.* 1985;27:958-960. **5.** Data on file, Stuart Pharmaceuticals, Wilmington, Delaware. **6.** Souza Dias MB, Jacobus NV, Tally FP, Gorbach SL. Activity of cefotetan against anaerobic bacteria. *Diagn Microbiol Infect Dis.* 1986;4:359-363. **7.** Wexler HM, Finegold SM. In vitro activity of cefotetan compared with that of other antimicrobial agents against anaerobic bacteria. *Antimicrob Agents Chemother.* 1988;32:601-604. **8.** Hennessey TD. Cefotetan: a compilation of susceptibility data of clinical isolates. In: Lode H, Periti P, Strachan CJL, eds. *Cefotetan: A Long-Acting Antibiotic for Polymicrobial Infections.* New York, NY: Churchill Livingstone; 1985:14-22. **9.** Centers for Disease Control. 1989 sexually transmitted diseases treatment guidelines. *MMWR.* 1989;38(suppl S-8):1-43.

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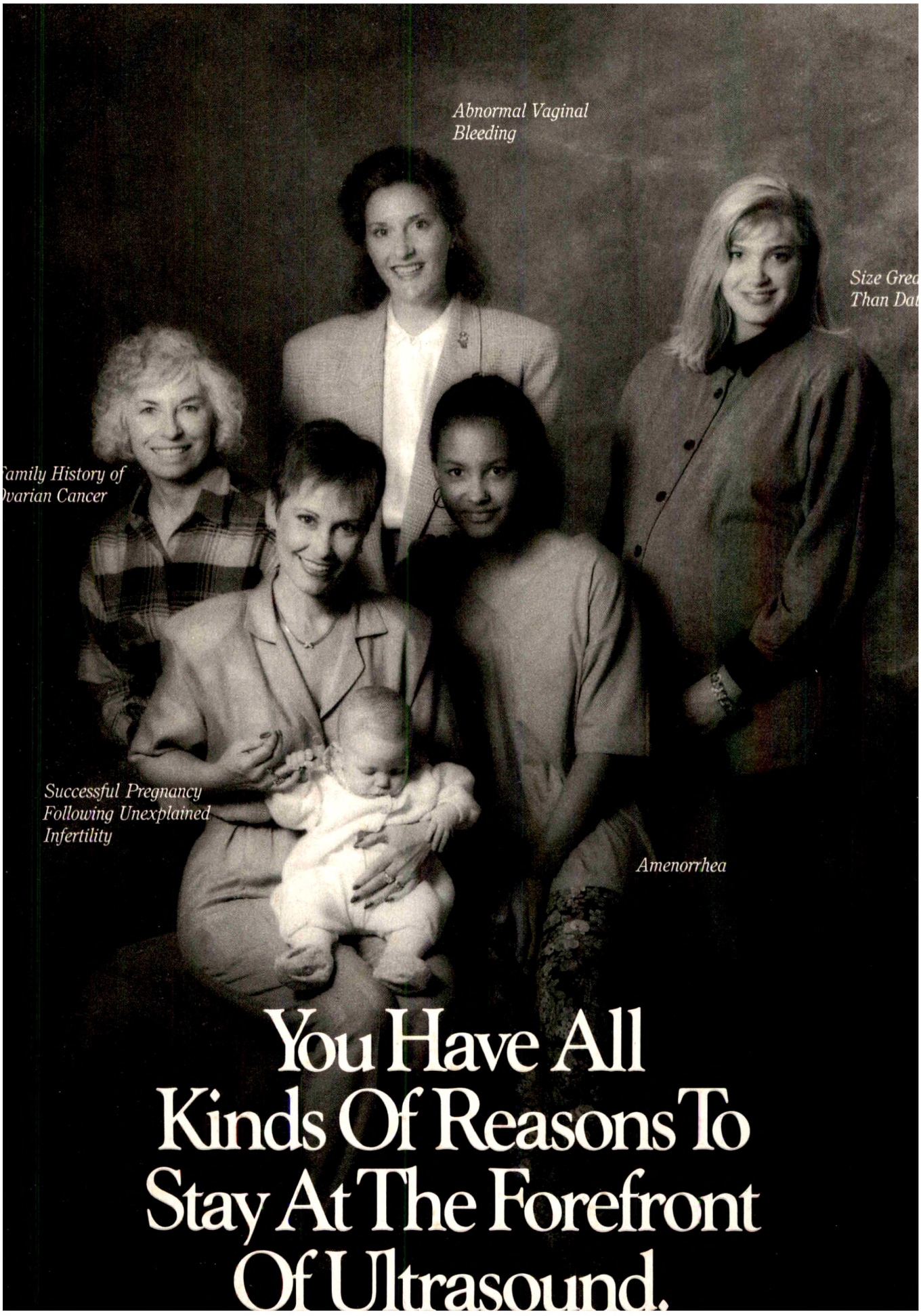
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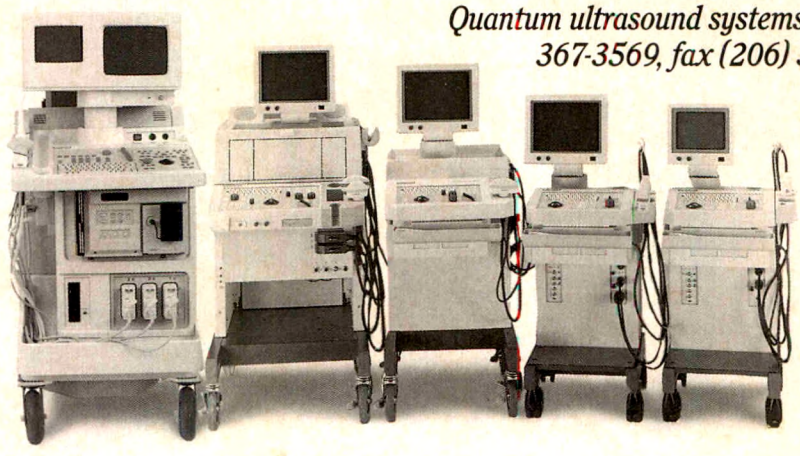
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—Guidelines for the care and use of *nonhuman* animals or *other species* approved by the institution have been followed as indicated in the *Material and Methods* section. *The species is named in the title, abstract, key words, and* Material and Methods *section.*
—The covering letter with essential information is with the manuscript.
—All elements of the manuscript are typed in English double-spaced on bond paper with 1-inch margins at top, bottom, and sides.
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"I'd like to have another child, but we also want some time before the next one."

Kris Miller
Clinton, MA



NORPLANT® SYSTEM
levonorgestrel implants

Lasts 5 years...yet is reversible

Serious as well as minor side effects may occur. The most common side effect which has been observed is menstrual bleeding irregularities.

Please see brief summary of prescribing information on the following page.

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NORPLANT® SYSTEM

levonorgestrel implants

Lasts five years…yet is reversible

BRIEF SUMMARY OF PRESCRIBING INFORMATION. CONSULT THE PACKAGE LITERATURE FOR FULL PRESCRIBING INFORMATION.

Indications and Usage

The NORPLANT SYSTEM is indicated for the prevention of pregnancy and is a long-term (up to 5 years) reversible contraceptive system. The capsules should be removed by the end of the 5th year. New capsules may be inserted at that time if continuing contraceptive protection is desired.

Contraindications

1. Active thrombophlebitis or thromboembolic disorders. 2. Undiagnosed abnormal genital bleeding. 3. Known or suspected pregnancy. 4. Acute liver disease; benign or malignant liver tumors. 5. Known or suspected carcinoma of the breast.

Warnings

A. WARNINGS BASED ON EXPERIENCE WITH THE NORPLANT SYSTEM

1. *Bleeding Irregularities* — Most women can expect some variation in menstrual bleeding patterns. Irregular menstrual bleeding, intermenstrual spotting, prolonged episodes of bleeding and spotting, and amenorrhea may occur, and could mask symptoms of cervical or endometrial cancer. Overall, these irregularities diminish with continued use. Because amenorrhea may occur, missed menstrual periods cannot serve as the only identifier of early pregnancy. Perform pregnancy tests whenever pregnancy is suspected. If pregnancy occurs, the capsules must be removed. Hemoglobin concentrations found in clinical trials generally indicated that reduced menstrual blood loss is associated with NORPLANT SYSTEM use. Blood loss resulting in hemoglobin values consistent with anemia occurred rarely.

2. *Delayed Follicular Atresia* — Atresia of the follicle is sometimes delayed, resulting in enlarged follicles that are clinically indistinguishable from ovarian cysts. In the majority of women, enlarged follicles disappear spontaneously. Rarely, they twist or rupture and surgical intervention may be required.

3. *Ectopic Pregnancies* — Ectopic pregnancies have occurred among NORPLANT SYSTEM users, although clinical studies have shown no increase in the rate of ectopic pregnancies per year among NORPLANT SYSTEM users as compared with users of no method or of IUDs. The incidence among NORPLANT SYSTEM users (1.3 per 1000 woman-years) was significantly below the rate estimated for noncontraceptive users in the U.S. (2.7 to 3.0 per 1000 woman-years). Ectopic pregnancy risk may increase with duration of NORPLANT SYSTEM use and increased weight of the user. Rule out ectopic pregnancy in any patient presenting with lower-abdominal pain.

4. *Breast-feeding* — Steroids are not the contraceptives of first choice for lactating women. Levonorgestrel has been identified in breast milk. Limited data suggests no significant effects on infant growth or health when mothers used the NORPLANT SYSTEM beginning 6 weeks after parturition.

5. *Foreign-body Carcinogenesis* — Rarely, cancers occur at foreign-body intrusion sites or old scars. None has been reported in NORPLANT SYSTEM clinical trials and risk to users is judged to be minimal.

6. *Thromboembolic Disorders* — Remove capsules if active thrombophlebitis or thromboembolic disease develops. With prolonged immobilization removal should be considered.

B. WARNINGS BASED ON EXPERIENCE WITH COMBINATION (PROGESTIN PLUS ESTROGEN) ORAL CONTRACEPTIVES (OCs)

Note: Many of the side effects or risks listed below are thought to be estrogen-related: the association of the NORPLANT SYSTEM progestin-only method to these risks is unknown.

1. *Cigarette Smoking* — Cigarette smoking increases the risk of serious cardiovascular side effects from combined OC use. Risk increases with age and heavy smoking (≥15 cigarettes/day) and is quite marked in women over 35 years old.

2. *Elevated Blood Pressure* — Increase in blood pressure has been reported in combination OC users; prevalence increases with long exposure.

3. *Thromboembolic Disorders and Other Vascular Problems* — An increased risk of thromboembolic and thrombotic disease is associated with combination OC use. Estimate of relative risk is 4- to 11-fold higher for users vs. nonusers.

Cerebrovascular Disorders: Combination OCs increase the relative and attributable risk of cerebrovascular events (thrombotic and hemorrhagic strokes). Generally, risk is greatest among hypertensive women > 35 years of age who smoke.

Myocardial Infarction (MI): An increased risk of MI has been attributed to combined OC use. This is thought to be primarily thrombotic in origin and related to the estrogen component. Increased risk occurs primarily in smokers or women with other underlying risk factors for coronary-artery disease. Relative risk of heart attack for combined OC users is estimated as 2 to 6 times that for nonusers. Absolute risk is very low for women under 30 years old.

Studies indicate a significant trend toward higher MI and stroke rates with increased progestin doses in combination OCs. However, recent data indicated no increased MI risk with past use of levonorgestrel-containing OCs.

4. *Carcinoma* — Recent evidence in the literature suggests no association between OC use and increased risk of breast cancer in the overall population of users. The Cancer and Steroid Hormone (CASH) study also showed no latent effect on breast cancer risk for at least a decade following long-term use. Some of these same studies have shown an increased relative risk of breast cancer in certain subgroups; no consistent pattern has been identified. Some studies suggest an association between combination OCs and an increase in the risk of cervical intra-epithelial neoplasia in some populations of women. The extent to which such findings may be due to differences in sexual behavior and other factors remains controversial. A cause-and-effect relationship between combined OC use and breast or cervical cancer has not been established. Combination OCs may decrease ovarian and endometrial cancer risk. Irregular bleeding patterns associated with NORPLANT SYSTEM use could mask cervical or endometrial cancer symptoms.

5. *Hepatic Tumors* — Hepatic adenomas are associated with combination OC use; estimated incidence is 3 events per 100,000 users per year. Risk increases after 4 or more years of use. Hepatic adenomas are benign but may rupture and cause death through intra-abdominal hemorrhage.

6. *Ocular Lesions* — Retinal thrombosis is associated with OC use and is believed to be related to the estrogen component. However, NORPLANT SYSTEM capsules should be removed if there is unexplained partial or complete vision loss; onset of proptosis or diplopia; papilledema; or retinal vascular lesions. Undertake appropriate diagnostic and therapeutic measures immediately.

7. *Use Before or During Early Pregnancy* — Extensive epidemiological studies reveal no increased risk of birth defects when OCs are used prior to pregnancy. Studies also do not suggest a teratogenic effect when taken inadvertently during early pregnancy. No evidence suggests that risk with NORPLANT SYSTEM use is different.

8. *Gallbladder Disease* — Early studies reported an increased lifetime relative risk of gallbladder surgery in OC or estrogen users. More recent studies, however, indicate that the relative risk of gallbladder disease with OC use may be minimal; this may be related to use of OCs with less estrogen and progestin content.

Precautions

GENERAL:

1. *Physical Examination and Follow-up* — A complete medical history and physical examination should be taken prior to implantation or reimplantation of NORPLANT SYSTEM capsules and at least annually during its use. Exams should include special reference to the implant site, blood pressure, breasts, abdomen and pelvic organs, including cervical cytology and relevant laboratory tests. Rule out malignancy in cases of undiagnosed, persistent or recurrent abnormal vaginal bleeding. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care.

2. *Carbohydrate Metabolism* — Altered glucose tolerance is found in some combination and progestin-only OC users. Effects of NORPLANT SYSTEM on carbohydrate metabolism appear minimal. Observe diabetic and prediabetic patients carefully while using the NORPLANT SYSTEM. Follow women being treated for hyperlipidemias closely if using the NORPLANT SYSTEM. Some progestins may elevate LDL and may render control of hyperlipidemias more difficult. (See **Warnings**.)

3. *Liver Function* — Consider removing capsules if jaundice develops. Steroid hormones may be poorly metabolized in patients with impaired liver function.

4. *Fluid Retention* — Steroid contraceptives may cause some degree of fluid retention. Prescribe with caution, and careful monitoring, in patients with conditions possibly aggravated by fluid retention.

5. *Emotional Disorders* — Consider removing capsules if significant depression occurs since the symptom may be drug-related. Observe carefully those with history of depression and consider removal if depression recurs to a serious degree.

6. *Contact Lenses* — Contact-lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist.

7. *Insertion and Removal* — Insertion is advised during the first 7 days of the cycle or immediately following abortion to insure that the woman is not pregnant and to assure contraceptive effectiveness during first cycle of use. Capsules may be inserted at any time during the cycle provided pregnancy has been excluded and a nonhormonal contraceptive method is used for the remainder of the cycle. Insertion is not recommended before 6 weeks postpartum in breast-feeding women. Follow insertion and removal instructions closely. Healthcare professionals are strongly advised to be instructed in the procedures before they attempt them. Proper insertion just under the skin facilitates removals; proper insertion and removal should result in minimal scarring. If all capsules cannot be removed at first attempt, attempt removal later when the site has healed. Bruising may occur at implant site during insertion or removal. Hyperpigmentation may occur at implant site but is usually reversible following removal. **See Full Prescribing Information for Detailed Insertion/Removal Instructions.**

8. *Infections* — Implant site infection has been uncommon (0.7%); aseptic technique and proper insertion/removal reduces possibility of infection. Institute treatment if infection occurs; remove capsules if infection persists.

9. *Expulsion* — Expulsion of capsules was uncommon; frequency increased when capsule placement was extremely shallow, was too close to incision, or when infection was present. Replace expelled capsule with new sterile capsule. Treat and cure any infection before replacement. Contraceptive efficacy may be inadequate with fewer than 6 capsules.

10. *Provisions for Removal* — Advise women that capsules may be removed at any time for any reason. Personnel instructed in removal technique should perform removal on request or at the end of 5 years of usage. Upon removal, dispose of capsules in accordance with Centers for Disease Control Guidelines for biohazardous waste.

DRUG INTERACTIONS: Reduced efficacy (pregnancy) in NORPLANT SYSTEM users has been reported when phenytoin or carbamazepine were used concomitantly. Warn NORPLANT SYSTEM users of possible decreased efficacy with use of related drugs.

DRUG/LABORATORY TEST INTERACTIONS: 1. Sex-hormone-binding globulin concentrations are decreased. 2. Thyroxine concentrations may be slightly decreased and triiodothyronine uptake increased.

CARCINOGENESIS: See **Warnings** section and Full Prescribing Information.

PREGNANCY: Pregnancy Category X. See **Warnings** section and Full Prescribing Information.

NURSING MOTHERS: See **Warnings** section and Full Prescribing Information.

INFORMATION FOR THE PATIENT: See Patient Labeling. Provide copy of patient labeling to the patient. Advise patients that Prescribing Information is available upon request. Inform prospective users of risks and benefits associated with NORPLANT SYSTEM use, with other forms of contraception, with no contraception, and about insertion/removal procedures. Informed consent from all patients may be desired in light of techniques involved with insertion and removal.

Adverse Reactions

The following have been associated with the NORPLANT SYSTEM during first year of use: many bleeding days or prolonged bleeding (27.6%); spotting (17.1%); amenorrhea (9.4%); irregular (onsets of) bleeding (7.6%); frequent bleeding onsets (7.0%); scanty bleeding (5.2%); pain or itching near implant site - usually transient - (3.7%); infection at implant site (0.7%); removal difficulties affecting subjects - based on 849 removals - (6.2%).

Controlled clinical studies suggest that the following, occurring during the first year, are probably associated with NORPLANT SYSTEM use: headache; nervousness; nausea; dizziness; adnexal enlargement; dermatitis; acne; change of appetite; mastalgia; weight gain; hirsutism, hypertrichosis and scalp-hair loss. The following were reported with a frequency of 5% or greater during the first year and possibly may be related to NORPLANT SYSTEM use: breast discharge; cervicitis; musculoskeletal pain; abdominal discomfort; leukorrhea; vaginitis.

Overdosage

Overdosage may cause fluid retention with its associated effects and uterine bleeding irregularities.

Dosage and Administration

The NORPLANT SYSTEM consists of six Silastic® capsules, each containing 36 mg of the progestin levonorgestrel. The total administered (implanted) dose is 216 mg. Implantation of all six capsules should be performed during the first 7 days of the onset of menses by a healthcare professional instructed in the NORPLANT SYSTEM insertion technique. Insertion is subdermal in the midportion of the upper arm about 8 to 10 cm above the elbow crease. Distribution should be in a fanlike pattern, about 15 degrees apart, for a total of 75 degrees. Proper insertion will facilitate later removal. **(See Full Prescribing Information for Detailed Insertion/Removal Instructions.)**

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TRANSACTIONS OF THE FIFTY-EIGHTH ANNUAL MEETING OF THE PACIFIC COAST OBSTETRICAL AND GYNECOLOGICAL SOCIETY

Folly, woodenheadedness, and the alluvial mind

Presidential address

Richard N. Bolton, MD

Portland, Oregon

One of my favorite authors wrote about history. If you think history is dull, I invite you to read any of Barbara W. Tuchman's books. They are as historic nonfiction should be—well documented, concise, exciting, and filled with the kind of insight that makes the past actions of mankind very much alive.

In my view, her finest book is *The March of Folly.*[1] Folly, she contends, "is the pursuit by governments of policy contrary to their own self-interest." Self-interest is composed of those ideas and policies that benefit the body being governed; folly is "policy which is counter productive." To qualify as folly, a policy must meet three criteria: First, "it must have been perceived as counter-productive in its own time"; second, "a feasible alternative course of action must have been available," and third, "the policy in question should be that of the group, not an individual."[2]

In *The March of Folly,* Tuchman details some prime examples: the Trojan horse, how George III of England and his advisers lost America, the excesses of the Renaissance popes that added fuel to the fire of reformation, the American involvement in a foolish, pointless war in Vietman, Louis XIV's revocation of the Edict of Nantes and the resulting persecution of the Huguenots, and the failure of Rehoboam, King of Israel, to repeal the heavy taxes imposed by his father, King Solomon. This folly in 930 BC resulted in the loss of the 10 northern tribes, destroying a kingdom put together by King David that had extended from northern Syria to Egypt. The emnity thus produced has caused war after war for 2800 years. The United States has just spent lives and a billion dollars a day fighting a new chapter in this same war and it is not over yet.

Regrettably, folly fueled by woodenheadedness is still with us. "Wooden-headedness is accessing a situation in terms of pre-conceived fixed notions while ignoring or rejecting contrary signs. It is acting according to wish while not allowing oneself to be deflected by the facts."[3] In other words, do not confuse me with facts, for my mind is made up. Woodenheadedness is alive and in full flower in Oregon. The governor of Oregon supported a specific tax intended to finance the increased cost of Medicaid. This tax was to be levied only on health care providers, totally ignoring societal responsibility. This same foolish concept could be applied to rent, automobiles, food, fire, police, bureaucrats, and lawyers. Happily, it did not get out of committee.

Another concept that helps explain curious political action is the "alluvial mind" "In such a mind, an idea develops so slowly that it hardly seems to have been there at all until it seems to have been there always."[4] The alluvial mind concept is described by William Irvine in his delightful book, *Apes, Angels and Victorians.*[4] This book is about Darwin, Huxley, and evolution. He describes Charles Darwin's concept of evolution as being alluvial with the whole having being derived from "tiny sands of evidence gradually accumulated during his long voyage on the *Beagle.*" This thought process is not geologic but rather the slow development of a concept until it is so engrained that its validity cannot be questioned.

The Supreme Court Title X "gag rule" decision seems a case in point. Family planning clinics accepting federal funds cannot mention abortion as a medical option. Ignore that this tramples on freedom of speech as guaranteed by the First Amendment. Ignore that

From The Womens Clinic.

Presented at the Fifty-eighth Annual Meeting of the Pacific Coast Obstetrical and Gynecological Society, Ashland, Oregon, September 9-12, 1991.

Reprints not available.

6/6/37158

this prevents health care workers from doing the best possible job of providing medically complete information. Ignore that this ruling comes awfully close to destroying the concept of separation of church and state; when life begins is a theologic argument and very difficult to document scientifically. Ignore the administrations' possible political pressure on the court; this pressure may have influenced this decision. Rather, focus on the term "federal funds." I submit that federal funds is an alluvial mind concept. There are no federal funds. Our government does not generate wealth. If it did, federal interest obligations would not be about $560 million a day,[5] and our 1991 federal deficit would not be $309 billion—the largest ever. Our government runs on red ink, and federal funds are our taxes. It would be nice if the citizen's funds were spent in a socially responsible manner.

Trade union, business, and government involvement in job-linked health insurance was born by accident during World War II.[6] The War Labor Board, to avoid inflation, put a ceiling on wages. So trade unions proposed "hidden" pay increases in the form of health insurance. The board gave approval and the alluvial mind concept of health insurance as a right was born.

Our legislatures have a real love-hate relationship with health care. This dilemma arises from the lure of office, fueled by the all-important need for reelection. The guiding principle in pursuing reelection is to "please as many and offend as few as possible."[7] Health care legislation is a positive source of votes; realistic health care funding, however, means higher taxes and will lose votes, a concept abhorrent to all politicians. There is no other way to explain the Veterans Administration Medical Service, the repeal of Medicare's catastrophic insurance, the underfunding of Medicaid, the Health Care Financing Administration's mauling of Medicare reimbursement, the emotions that have blocked investigation of RU 486 for nonabortion use, and the general silliness that has made acquired immunodeficiency syndrome a political disease instead of an infectious disease.

For these and many other reasons, the citizens of the United States cannot afford more politically motivated government meddling in health care. There is too much room for folly, woodenheadedness, and the alluvial mind. Folly and health care can be avoided. I sincerely urge you all to consider the American Medical Association's proposal for affordable quality health care: Health Access America.[8] It can be made to work for all and does not destroy the best health care in the world. Its salient points are as follows: (1) Improvements to the American health care system should preserve the strengths of our current system; (2) affordable coverage for appropriate health care should be available to all Americans regardless of income; (3) particular efforts are needed to assure continued access by the elderly to affordable health care services; (4) health care services should be delivered with high quality and appropriate costs; (5) patients should be free to determine from whom and the manner in which health care benefits are delivered; (6) all physicians should be committed to the highest ethical standards in the delivery of care to patients.

In my conception a health care system funded by taxes and administered entirely by the federal government would have the compassion of the Internal Revenue Service, the efficiency of the post office, a budget to make the Pentagon blush, and the overall benevolence of the Bureau of Indian Affairs.

After all, what can you expect from a government that outlaws Lawn Darts but lets us keep handguns.

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Therapeutic donor insemination: A prospective randomized trial of fresh versus frozen sperm

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OBJECTIVE: We evaluated the efficacy of fresh versus frozen sperm in therapeutic donor insemination.
STUDY DESIGN: Fifty-seven women underwent 72 courses of treatment (a maximum of six therapeutic donor insemination cycles—three fresh and three frozen) totaling 198 cycles. Each woman served as her own control and was prospectively randomized to receive a single, timed insemination of either fresh or frozen sperm.
RESULTS: Fecundity was 20.6% for fresh sperm cycles and 9.4% for frozen ($p < 0.03$, by χ^2 analysis). Fresh cervical cap insemination fecundity was 20.3%; frozen was 7.8% ($p < 0.03$, by χ^2 analysis). Fresh intrauterine insemination fecundity was 21.2%; frozen was 15.8% ($p = 0.63$, by χ^2 analysis). Fresh 3-month life-table pregnancy rates were 48% ± 10%; frozen rates were 22% ± 8% ($p = 0.05$ by Breslow analysis). Survival analysis with fixed covariates showed a positive association with the use of fresh sperm ($p = 0.04$).
CONCLUSION: Cycle fecundity was significantly greater with fresh sperm in women undergoing cervical cap insemination or intrauterine insemination and in women undergoing only cervical cap insemination. These results have important implications for contemporary management of patients undergoing therapeutic donor insemination with frozen sperm. (Am J Obstet Gynecol 1992;166:1597-1606.)

Key words: Fresh sperm, frozen sperm, therapeutic donor insemination

The American Fertility Society amended its therapeutic donor insemination guidelines in February 1988, stating that " . . . the use of fresh semen for donor insemination is no longer warranted."[1, 2] This statement responded to concern about human immunodeficiency virus (HIV) transmission in therapeutic donor insemination. Under the new guidelines only sperm from donors who are seronegative for HIV 6 months after sperm donation and freezing would be used. This change of guidelines has been controversial since many physicians believe that the use of fresh sperm results in increased pregnancy rates and/or fecundity as compared with those for frozen (cryopreserved) sperm. In addition, the risk of HIV infection after one insemination with fresh sperm from a screened donor is exceedingly remote, being approximately 1 in 500 million.[3] However, published reports have documented HIV infection after therapeutic donor insemination with sperm from asymptomatic HIV-positive donors.[4, 5]

There are many viral, bacterial, and parasitic pathogens in semen, and transmission of infectious agents has been reported in fresh semen.[6] The effects of freezing on these pathogens are not well documented, but freezing does not completely inactivate HIV, herpes simplex virus, cytomegalovirus, *Neisseria gonorrhoeae, Chlamydia trachomatis,* or the mycoplasmas.[7] The advantages of frozen semen include a quarantine period in storage with donor retesting before insemination. For HIV, this permits testing for proviral deoxyribonucleic acid with a polymerase chain reaction technique and testing for serum antibodies, neither of which may be in high enough concentrations for detection at the time of donation. Freezing of semen may also reduce the concentration of some pathogens.[6] Frozen sperm allows insemination when timing is optimal, multiple inseminations in a single cycle, and the use of the same donor for subsequent pregnancies.[8] Disadvantages of frozen sperm include a possibly decreased pregnancy rate and/or cycle fecundity and possibly more inseminations per cycle as compared with fresh sperm.[9-14] The decreased fecundity of frozen sperm is due, in part, to structural and functional damage resulting from the freezing process.[8, 9, 15, 16]

Few randomized, controlled comparisons of fresh versus frozen sperm in therapeutic donor insemination have been done, and ongoing trials with large patient

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populations and current cryopreservation techniques are needed.[8, 11, 12, 17] This study compares the efficacy of fresh and frozen sperm in therapeutic donor insemination in a prospective, randomized clinical trial with each woman serving as her own control.

Material and methods

Patient selection and treatment. Couples presenting for therapeutic donor insemination at a referral infertility practice between June 1988 and December 1990 were given the option of receiving only frozen sperm or participating in this study and receiving randomized fresh and frozen sperm. Couples were thoroughly counseled of their options and the risks of fresh therapeutic donor insemination. This study was approved by the Stanford Committee for the Protection of Human Subjects in Research. All couples had male factor as one cause of infertility and underwent infertility screening consisting of basal body temperature graphing, urinary luteinizing hormone (LH) assay, serum progesterone measurement, cervical mucus evaluation, semen analysis, and *C. trachomatis* and mycoplasma cultures. Endometrial biopsy, postcoital test, hamster egg penetration assay, hysterosalpingography, laparoscopy, and laparotomy were performed as clinically indicated. Women had at least one patent fallopian tube and were ovulating either spontaneously or with clomiphene citrate or human menopausal gonadotropins (or both). Ovulatory dysfunction was defined as menstrual cycles <21 or >35 days in length or an abnormal luteal phase (two endometrial biopsy specimens >2 days out of phase). Endometriosis and adhesions were treated with carbon dioxide laser vaporization at surgery.

Couples agreed to six cycles of therapeutic donor insemination—three cycles with fresh and three cycles with frozen sperm. A course of treatment was defined as a maximum of six cycles of therapeutic donor insemination and was terminated by conception or withdrawal from the study. Insemination type (fresh or frozen) was prospectively assigned by random number tables, such that no more than two consecutive inseminations with either fresh or frozen sperm were performed. The protocol was established so that all fresh and frozen inseminations for a given patient used sperm from the same donor. In only five courses of treatment was it necessary at any time to use frozen sperm from a different donor. Women were not aware of the type of sperm they were receiving. A single insemination per cycle with either fresh or frozen sperm was performed within 12 to 36 hours of the LH surge with either cervical cap insemination or intrauterine insemination. Whole ejaculate was used for cervical cap insemination with fresh sperm, and 1 ml of thawed whole ejaculate was used for cervical cap insemination with frozen sperm. For intrauterine insemination a maximum of 0.5 ml of washed sperm was slowly infused with a 1 ml syringe and a 3.5 Fr TomCat catheter (Sherwood Medical, St. Louis) or a Makler cannula (Sefi Medical Instruments, Haifa). Women remained supine for 20 minutes with the cannula in place. They were given oral doxycycline (100 mg) the evening before intrauterine insemination as prophylaxis against infection. Indications for intrauterine insemination included poor cervical mucus determined by abnormal postcoital test (actively motile sperm <1/high-power field) or abnormal cervical mucus penetration test (<25 mm in 90 minutes), female serum Immunobead antisperm antibodies, and high-dose clomiphene citrate ovulation stimulation (at least 100 mg/day). Timing of ovulation was monitored with basal body temperature charts and LH urinary monoclonal antibody home testing (OvuQUICK, Monoclonal Antibodies, Mountain View, Calif.). If menses did not occur, a quantitative β-human chorionic gonadotropin (β-hCG) assay was done and repeated in 2 days to confirm pregnancy. If women did not conceive during their first course of treatment (six insemination cycles), they were given the option, during a second course of treatment, to enter the study again with the same donor or a different one. When women conceived on a nonprotocol cycle, they were terminated from the study with the status "not pregnant." When women had an insemination on a nonprotocol cycle, they continued on the same study "course of treatment" with appropriate randomization in the next cycle. When a women conceived on a study protocol cycle, she was terminated from the study with the status "pregnant." After spontaneous abortion or delivery, women were reentered in a new "course of treatment" with randomized inseminations beginning with cycle 1. Data were recoded for the subsequent cycle to reflect changes in parameters such as age, gravidity, and duration of infertility.

Sperm preparation. Fresh ejaculates were allowed to liquefy for 30 minutes at 37° C, then suspended in a 1:1 ratio with TEST-yolk buffer with glycerol (Irvine Scientific, Santa Ana, Calif.) cryoprotectant. Vials containing 1.8 ml were divided into aliquots and frozen in a programmable freezer (Planar Products, Ltd., Middlesex, England) at the Fertility and Reproductive Health Institute of Northern California. Semen was cooled at −0.5° C per minute to −5° C. After holding for 10 minutes, semen was rapidly cooled at −10° C per minute to −80° C, held for 10 minutes, and stored in liquid nitrogen. Specimens were thawed at room temperature and washed or used for insemination within minutes of thawing. Sperm used for intrauterine

insemination were washed twice with a modified human tubal fluid medium (Irvine Scientific). After the second wash, the sperm pellet was suspended in 0.5 ml of medium and allowed to swim up in a standard procedure.

Standard semen analyses were performed before freezing and before and after washing. Semen analyses were not done in fresh cervical cap insemination cycles. Sperm parameters reported were from the donor's most recent semen analysis. For cervical cap insemination with frozen sperm, samples were evaluated microscopically under high power to assure sperm movement after thawing, but a semen analysis was not done. To estimate postthaw sperm parameters, we multiplied prefreeze sperm parameters by the average decrease in count and motility observed in intrauterine insemination cycles with frozen sperm (prefreeze and postfreeze semen analyses were performed). Azoospermia was defined as the absence of sperm, oligospermia as sperm count $<20 \times 10^6$/ml, and asthenospermia as <30% progressively motile sperm.

Donor selection. Sperm from seven donors was used in this study. American Fertility Society guidelines for selecting and screening donors were followed.[1] We required that donors have two normal semen analyses (count $>60 \times 10^6$/ml, progressive motility >30%, total motility >50%, and >60% normal morphologic features) and excellent sperm recovery after freezing and thawing ($>100 \times 10^6$ actively motile sperm). They then underwent investigation including blood typing, complete blood count, serum chemistry tests, sedimentation rate, and serologic testing for HIV (two tests, 5 to 6 weeks apart), syphilis (Venereal Disease Research Laboratory test), hepatitis profile, cytomegalovirus, and HSV type II antibodies. Urethral cultures for gonorrhea, chlamydia, mycoplasma, and other pathogens were done. HIV testing was also done with a polymerase chain reaction gene amplification method. Donors underwent a thorough history and physical examination, genetic screening, and karyotyping by a geneticist. Donors must have had no genetic predisposition for heritable disease, no more than one sexual partner within the past year, and no history of male sexual partners or alcohol, illicit drug, or intravenous drug abuse. Monthly HIV and other sexually transmitted disease tests were done; this regimen was more frequent and rigorous than recommended.[1]

Data analysis. Statistical analyses were performed with BMDP Statistical Software, Inc. (Los Angeles) and SAS (SAS Institute, Inc., Cary, N.C.). Analysis included Student's *t*-tests, χ^2 tests, life-table calculations, and survival analyses with fixed covariates according to the Cox model. Cycle fecundity or the probability of conception per cycle was calculated by the method of Cramer et al.[18] Because 13 women underwent more than one course of treatment, analyses were performed on courses of treatment rather than on patients. Values are expressed as mean ± SE and range.

Results

Patient characteristics. Fifty-seven women entered the study and underwent 72 courses of treatment. Forty-four women began one, 11 began two, and two began three courses of treatment. In five women the first six cycles did not result in pregnancy and they continued the treatment regimen, with one becoming pregnant. Three were treated for five or six cycles, conceived while off protocol, and returned for a second course of treatment, during which all three conceived. Three conceived while on protocol and returned for a second course of treatment. Of the two women with three courses of treatment, the first did not conceive in 6 months and had a second course of treatment resulting in a spontaneous abortion and a third resulting in a live birth. The second patient had six cycles without pregnancy, conceived while off protocol and had a spontaneous abortion, then conceived while on protocol and had a spontaneous abortion, and again conceived while on protocol and had a live birth.

Women were 32.5 ± 0.5 (range 22 to 42) years old with 5.2 ± 0.6 (range 0.1 to 9.7) years of infertility. Twenty-nine (40.3%) were nulligravid and 36 (50%) male partners had never fathered a child. Female infertility factors included 16 (22.2%) women with ovulatory dysfunction, 20 (27.8%) with endometriosis, 4 (5.6%) with unilateral tubal occlusion or fimbrial damage, and 4 (5.6%) with cervical factor infertility (poor cervical mucus or female antisperm antibodies). Thirty-eight (52.8%) women had no identifiable infertility factor. Forty-five (62.5%) men were azoospermic and 30 (41.7%) had had a vasectomy. Twenty-two of 30 (73.3%) men had attempted reversal of vasectomy and 7 of 8 (87.5%) men underwent varicocele repair but were still unable to conceive.

Outcome. There were 30 conceptions during 198 insemination cycles for a fecundity of 15.2% (Table I). Eight women conceived in nonprotocol cycles. Forty-seven percent ($n = 14$) of conceptions occurred in the first insemination cycle, 77% ($n = 23$) within two cycles, and 83% ($n = 25$) within three cycles. Conceptions resulted in 21 live births and nine spontaneous abortions (six in fresh and three in frozen insemination cycles). Twenty-one conceptions occurred in 102 insemination cycles with fresh sperm (fecundity = 20.6%), and nine conceptions took place in 96 insemination cycles with frozen sperm (fecundity = 9.4%, $p < 0.03$ by χ^2 analysis). Women achieved 20 pregnancies in 146 cycles of

Table I. Cycle fecundity by type of insemination

Type of insemination	*No. of cycles*	*No. of conceptions*	*Cycle fecundity (%)*
Fresh sperm	102	21	20.6*
Frozen sperm	96	9	9.4
Total	198	30	15.2
Cervical cap insemination			
Total	146	20	13.7
Fresh sperm	69	14	20.3*
Frozen sperm	77	6	7.8
Intrauterine insemination			
Total	52	10	19.2
Fresh sperm	33	7	21.2
Frozen sperm	19	3	15.8

*$p < 0.03$ for fresh versus frozen sperm by χ^2 analysis.

cervical cap insemination (fecundity = 13.7%) and 10 pregnancies in 52 cycles of intrauterine insemination (fecundity = 19.2%, $p = 0.34$). Fecundity was similar in fresh cervical cap insemination (20.3%) and fresh intrauterine insemination (21.2%) cycles ($p = 0.91$) and in frozen cervical cap insemination (7.8%) and frozen intrauterine insemination (15.8%) cycles ($p = 0.28$). Cycle fecundity was significantly greater in fresh than in frozen cervical cap insemination cycles ($p < 0.03$) but was similar for fresh and frozen intrauterine insemination cycles ($p = 0.63$). Ovulation stimulation was performed in 44 (84.6%) intrauterine insemination and 6 (4.1%) cervical cap insemination cycles ($p = 0.000$). There were no ectopic pregnancies, pelvic infections, or complications during the course of the study.

We examined the effect of known female infertility factors on fecundity in women receiving both insemination types (fresh or frozen sperm) and insemination methods (cervical cap insemination or intrauterine insemination, Table II). The greatest fecundity was observed in women with cervical factor infertility (fecundity = 44.4%). This fecundity was significantly greater than that for women without cervical factor infertility ($p = 0.03$). Most women with cervical factor infertility received intrauterine insemination, which was also associated with greater cycle fecundity in this group ($p = 0.01$ for intrauterine insemination vs cervical cap insemination). However, the number of women with cervical factor infertility was very small. Women with no identified infertility factors and women with azoospermic male partners had fecundity similar to that of others. The lowest fecundity was in women with tubal damage.

Life-table estimated cumulative pregnancy rates at 3 months (± SE) were 0.48 ± 0.10 after insemination with frest sperm and 0.22 ± 0.08 after insemination with frozen sperm (Fig. 1, $p = 0.05$ by Breslow analysis, $p = 0.03$ by Mantel-Cox analysis). Three-month estimated cumulative pregnancy rates were 0.55 ± 0.14 for cervical cap insemination with fresh sperm, 0.23 ± 0.10 for cervical cap insemination with frozen sperm, 0.33 ± 0.10 for intrauterine insemination with fresh sperm, and 0.22 ± 0.11 for intrauterine insemination with frozen sperm (Fig. 2). Estimated cumulative pregnancy rates at 3 months were greater for cervical cap insemination with fresh sperm than with frozen sperm ($p = 0.05$ by Breslow analysis, $p = 0.03$ by Mantel-Cox analysis) but similar for intrauterine insemination with fresh versus frozen sperm ($p = 0.57$ by Breslow analysis, $p = 0.57$ by Mantel-Cox analysis).

Survival analyses evaluated the effect of single variables on the probability of conception. Cervical factor infertility ($p = 0.03$) and more extensive adhesions staged according to the American Fertility Society revised 1985 scoring system ($p = 0.04$) were positively associated with pregnancy. Fresh sperm was positively associated with probability of pregnancy in cervical cap insemination and intrauterine insemination cycles ($p = 0.04$) and in cervical cap insemination only cycles ($p = 0.04$). Fresh sperm was not associated with pregnancy in intrauterine insemination only cycles ($p = 0.52$). In addition, intrauterine insemination (vs cervical cap insemination) was not positively associated with pregnancy.

Semen analyses. An average of 262.6 ± 15.7 × 10^6 motile sperm (Volume × Count × Progressive motility/100) were used for fresh cervical cap insemination, 22.7 ± 1.2 × 10^6 for frozen cervical cap insemination, 32.6 ± 3.9 × 10^6 for fresh intrauterine insemination, and 5.6 ± 1.1 × 10^6 for frozen intrauterine insemination. We did not observe a significant association between number of motile sperm inseminated and fecundity in any of the insemination groups by use of *t* tests and survival analyses with fixed covariates (Table III). With freezing, sperm count decreased by an average of 60% ± 5% and progressive motility decreased by 63% ± 4%.

Donors were ranked from highest to lowest fecundity. Only one donor had significantly greater fecundity with fresh (fecundity = 0.30) than with frozen (fecundity = 0.05) insemination cycles ($p < 0.04$). For donors with >10 insemination cycles, there was no difference in fecundity between the three donors with the greatest and the two with the lowest ($p < 0.15$). No donor tested positive for HIV.

Comment

Prospective randomized clinical trials have shown that frozen sperm result in similar cumulative pregnancy rates over a 1-year period (45% to 73%) when compared with those of fresh sperm (53% to 97%) but may have decreased cycle fecundity, as compared with fresh sperm.[8, 11, 12, 17] Studies in which only fresh sperm was used show a fecundity of 15% versus 5% to 11% with frozen sperm.[12, 14, 19-22] Two of three prospective

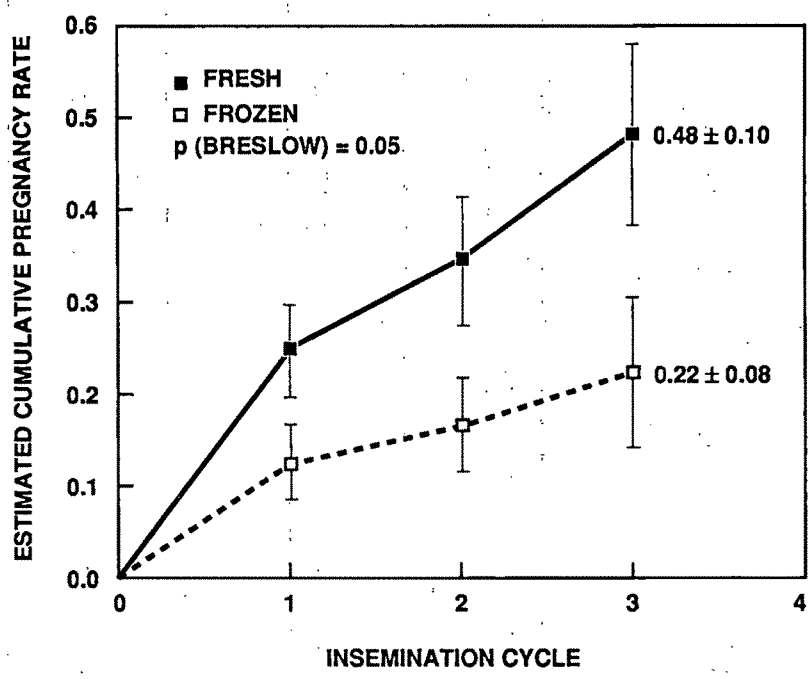


Fig. 1. Estimated life-table cumulative pregnancy rates at 1-month intervals. Estimated cumulative pregnancy rates over 3 months were greater for fresh than frozen insemination cycles ($p = 0.05$ by Breslow analysis). *Bars* indicate standard error.

Table II. Infertility factors and fecundity after cervical cap insemination or intrauterine insemination with fresh or frozen donor sperm

	All cycles		*Fresh cervical cap insemination*		*Frozen cervical cap insemination*		*Fresh intrauterine insemination*		*Frozen intrauterine insemination*	
Cause of infertility	*No. of cycles*	*No. pregnant*	*No. of cycles*	*No. pregnant*	*No. of cycles*	*No. pregnant*	*No. of cycles*	*No. pregnant*	*No. of cycles*	*No. pregnant*
No female factor	109	16 (15)	47	11 (23)	51	5 (10)	7	0 (0)	4	0 (0)
Ovulatory disorder	41	7 (17)	10	1 (10)	14	1 (7)	12	4 (33)	5	1 (20)
Cervical factor	9	4 (44)*	1	0 (0)	4	0 (0)	3	3 (100)†	1	1 (100)
Endometriosis	49	6 (12)	12	2 (17)	12	0 (0)	15	2 (13)	10	2 (20)
Tubal damage	8	0 (0)	2	0 (0)	3	0 (0)	4	0 (0)	1	0 (0)
Azoospermic male	121	20 (16)	41	11 (27)	48	3 (6)	20	3 (15)	12	3 (25)

Values in parentheses are fecundity rates.

*$p = 0.03$, versus cycles in women without cervical factor.

†$p = 0.01$, versus fresh intrauterine insemination cycles in women without cervical factor.

randomized studies using women as their own controls observed a threefold to fourfold increase in cycle fecundity ($p < 0.001$) with similar 1-year estimated cumulative pregnancy rates for fresh compared with frozen sperm.[11, 12] We observed fecundity similar to that reported in the literature for fresh (20.6%) and frozen (9.4%) insemination cycles and a twofold increase in 3-month estimated cumulative pregnancy rates with fresh sperm compared with frozen sperm. This increased pregnancy rate with fresh sperm occurred in cervical cap insemination cycles but not intrauterine insemination cycles.

Decreases in fecundity and cumulative pregnancies have been attributed to sperm damage during processing and freezing and fewer total motile sperm used per insemination.[15] Such damage may be less important in intrauterine insemination cycles because sperm are deposited beyond the barrier of the cervix and more proximal to the oocyte. Similar fecundity rates for fresh and frozen sperm have been observed for intrauterine insemination and in vitro fertilization, insemination techniques that bypass many of the obstacles sperm must overcome with cervical cap insemination.[23]

Studies have shown that the great majority of patients

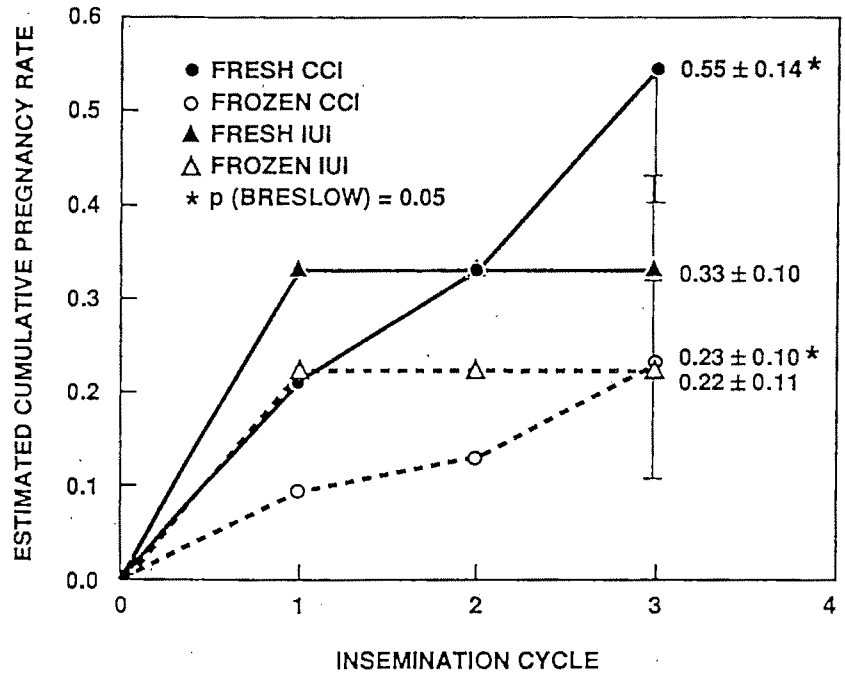


Fig. 2. Estimated life-table cumulative pregnancy rates at 1-month intervals. Estimated cumulative pregnancy rates over 3 months were greater for cervical cap insemination with fresh sperm than for cervical cap insemination with frozen sperm ($p = 0.05$ by Breslow analysis). *Bars* indicate standard error.

Table III. Millions of motile sperm inseminated ± standard error with ranges in conception and nonconception cycles

Insemination type	*Conception cycle*	*Nonconception cycle*	*Significance*
Cervical cap insemination			
Fresh sperm	249 ± 32 (57-514) ($n = 14$)	266 ± 18 (18-621) ($n = 55$)	$p = 0.66$
Frozen sperm	43 ± 7 (20-65) ($n = 6$)	33 ± 2 (4-81) ($n = 71$)	$p = 0.18$
Intrauterine insemination			
Fresh sperm	42 ± 9 (17-87) ($n = 7$)	30 ± 4 (1-91) ($n = 26$)	$p = 0.24$
Frozen sperm	1.2 ± 0.5 (0.7-1.6) ($n = 2$)	6.1 ± 1.2 (0.6-15) ($n = 16$)	$p = 0.18$

who conceive with therapeutic donor insemination do so within six cycles.[10] In this study 83% of conceptions occurred within three insemination cycles and all intrauterine insemination conceptions occurred in the first cycle. Women failing to conceive after three cycles with cervical cap insemination may benefit from several intrauterine insemination cycles. Controlled ovarian hyperstimulation, with clomiphene citrate initially and then with human menopausal gonadotropin, also should be considered since this may increase fecundity further. In clinical practice 9 to 12 months of therapeutic donor insemination may be a sufficient trial before other fertilization methods or adoption is attempted.

Our spontaneous abortion rate of 29% after conceptions with fresh sperm and 33% after frozen were slightly higher than the reported range of 11% to 17%.[10, 15] This may be due to the age and/or infertility status of our patients or to our obtaining serum pregnancy test results at just 2 weeks after insemination.

Increasing age, duration of infertility, pelvic abnormalities, and oligospermia have been associated with significantly decreased therapeutic donor insemination pregnancy rates.[15, 24] The only factors we found to be positively associated with the probability of pregnancy were cervical factor infertility, treated adhesions, and use of fresh sperm. This association with cervical factor infertility was probably due to the successful use of

intrauterine insemination in this group, because women in all of four intrauterine insemination cycles conceived. Increased fecundity with treated adhesions is probably due to increased tubal and ovarian mobility.

Pregnancy rate and fecundity have been positively associated with the number of motile sperm inseminated, and there may be a critical number of sperm required to attain a maximal outcome.[8, 11, 15, 21] Bordson et al.[21] observed similar fecundity rates with intracervical insemination for both fresh and frozen sperm when at least 40×10^6 motile sperm were inseminated. Keel and Webster[8] found that frozen ejaculates with at least 20×10^6 motile sperm had fecundity rate similar to those for fresh ejaculates that contained an average of five times the number of motile sperm. Byrd et al.[15] found that the minimum number of motile sperm required to obtain optimal fecundity occurred in their arbitrarily assigned groups having between 6 and 15×10^6 for intrauterine insemination and between 50 and 100×10^6 for intracervical insemination with frozen sperm. For rates of number of motile sperm inseminated below these values, fecundity was positively associated with number of motile sperm inseminated. We did not find an association between number of motile sperm inseminated and pregnancy, probably because of an inadequate number of cycles. Also, values for sperm parameters in fresh and frozen cervical cap insemination cycles were estimated and therefore are of uncertain accuracy.

The optimal time for insemination with fresh and frozen sperm may be different. Frozen sperm have decreased motility and viability, requiring more careful timing of insemination and perhaps necessitating more inseminations per cycle and increased cost than fresh sperm.[15, 16] In addition, the optimal time for insemination with frozen sperm may be different for intrauterine insemination than for cervical insemination. The highest cycle fecundity with cervical cap insemination with frozen sperm was achieved when inseminations were performed 0 to 10 hours after the LH peak versus 10 to 20 hours with intrauterine insemination.[15] We timed intrauterine insemination and cervical cap insemination with urinary LH home testing and basal body temperature graphs. In addition, women with ovulatory dysfunction had ovulation stimulation with clomiphene citrate with or without human menopausal gonadotropin. Ovulation stimulation allowed more accurate timing of insemination. In studies that compared single and multiple inseminations per cycle with intracervical insemination, fecundity was not affected by the number of inseminations per cycle.[17, 21] However, Byrd et al.[15] found an increase in fecundity when two inseminations per cycle were done with intrauterine insemination and frozen sperm.

Donor retesting for HIV 6 months after sperm donation and freezing is recommended because the virus can be transmitted and is infectious in fresh and cryopreserved sperm.[4] The risks of HIV infection after exposure in therapeutic donor insemination are unknown. In this study no donor tested positive for HIV. In other studies seven women have been reported to be seropositive for HIV after therapeutic donor insemination with an HIV-positive donor.[4, 5] However, women's HIV status was not documented before therapeutic donor insemination cycles. The risk of HIV infection after a single episode of vaginal intercourse with an infected partner is estimated as ≤ 0.002.[3] When the partner is known to be HIV negative and has no history of high-risk behavior, the risk of infection after one episode of unprotected intercourse is estimated to be one in 500,000,000.[5] Using a carefully screened, reliable donor pool and frequent HIV testing with polymerase chain reaction should make the risk of HIV transmission in therapeutic donor insemination with fresh sperm exceedingly remote. Polymerase chain reaction is a highly sensitive test for HIV proviral deoxyribonucleic acid that detects infection earlier than tests for serum HIV antibodies and viral culture.[25]

Fresh sperm is believed to be more effective, thereby requiring less time and cost to conceive than frozen sperm.[9] The eventual percentage of couples conceiving could be similar for fresh and frozen sperm, but frozen sperm requires up to double the time to conceive because the pregnancy rate is less.[13, 14] In this study insemination with fresh sperm resulted in greater cycle fecundity ($p < 0.03$), life-table estimated 3-month cumulative pregnancy rates ($p = 0.05$ by Breslow analysis), and positive association with pregnancy in survival analyses ($p = 0.04$). Nevertheless, given the current medicolegal and political status surrounding the use of fresh sperm, frozen sperm has become almost the universal standard. Therefore the reduced fecundity associated with therapeutic donor insemination with frozen sperm must be recognized in our counseling and management of couples undergoing therapeutic donor insemination.

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Discussion

DR. PHILIP E. YOUNG, San Diego, California. There is a great deal of information in this article, but I shall limit my comments to the major issues. (1) Is fecundity, the pregnancy rate per cycle, obtained with fresh semen different from that obtained with frozen semen? Is the difference large enough to be meaningful for patients who undergo three cycles of insemination? (2) Is the answer affected by whether the insemination is done vaginally or directly into the uterus?

The answers to these questions are important because the risk of HIV transmission with proper screening is very small, but it is not zero. If there is a substantial long-term better chance of pregnancy with fresh rather than frozen semen, there may well be a number of couples who would use fresh semen, opting to take the remote chance of HIV transmission to improve the chance of achieving a pregnancy. If, over the duration, the chance of pregnancy were the same for fresh and for frozen semen, it is difficult to imagine that anyone would choose use of fresh semen, regardless of how small the difference in risk of HIV transmission.

The questions addressed in this study have been discussed by many other studies, including one I presented to this society 3 years ago.[1] The major strengths of Dr. Adamson's study are that it was a prospective study and that it included a crossover design in which the subjects were assigned equal numbers of inseminations with fresh or frozen semen with the order randomly determined. This design has not, to my knowledge, been applied to this question before. Randomization is especially important because the group of patients studied was a disparate group, as the group of patients undergoing therapeutic donor insemination always is. Many of his subjects had several other potential causes of infertility.

The weaknesses of this study are as follows: (1) The study deals with a relatively small number of patients: 57 women undergoing 72 treatment cycles of six or fewer inseminations each, giving a total of 198 inseminations. (2) Semen analysis was done only periodically on the donors of the fresh specimens, not with each insemination. Further, semen analysis was not done on thawed specimens used for vaginal insemination. Thus, although the article contains a table comparing sperm counts for both fresh and frozen sperm with both intrauterine insemination and the vaginal inseminations, these counts are often estimated. The assumption of

stability in count is, perhaps, reasonable for clinical decisions but not for scientific analysis. Given these caveats, what answers do the authors provide?

They tell us that when semen is applied to the cervix the cycle fecundity of fresh semen is 20.6% versus 7.8% per cycle for frozen semen, a statistically significant difference. There is also a significant difference in the estimated cumulative probability of pregnancy at 3 months, that for fresh semen being 55% and that for frozen semen being 23%. This is the most meaningful conclusion of the article. When the authors combined the vaginal and intrauterine data, there was also a significant difference between the fresh and frozen groups as one might anticipate since the intrauterine group is relatively small.

When fresh and frozen sperm were compared for intrauterine inseminations alone, there was no significant difference. The authors concluded that in some way a defect present in frozen semen is overcome by the processes involved in intrauterine insemination, a conclusion I believe is not warranted by the data because of the small number of patients studied and the fact that this aspect of the treatment was not randomly assigned. Hence, the authors' discussions of possible reasons why fresh semen does not appear to be better than frozen for intrauterine insemination are quite speculative.

This article supports the conventional wisdom, and the majority of reports in the literature, that the cycle fecundity of fresh semen is substantially better than that of frozen semen. I do not agree that a 3-month evaluation of the cumulative probability of pregnancy is sufficient to assess the efficacy of frozen versus fresh semen. The literature suggests that follow-up extending to 9 or 12 months will often show only minimal differences between the cumulative probabilities of pregnancy for fresh and frozen semen.

It is clear that the use of frozen semen for therapeutic donor insemination is now the standard of care in the United States. Therefore I agree with the authors' final statement that the reduced fecundity associated with therapeutic donor insemination with frozen sperm must be recognized in our counseling and management of couples undergoing therapeutic donor insemination. I personally believe the major variable in the insemination process that affects the pregnancy rate is the total number of motile sperm delivered. I believe that as technology improves and this number increases for frozen semen, the time required to achieve a given cumulative probability of pregnancy with frozen specimens will be decreased.

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DR. KENNETH A. BURRY, Portland, Oregon. This is an excellent article. The experience at Oregon Health Sciences University in a prospective comparison of cycle fecundity with fresh and frozen semen is similar to that of the authors. Also similar are our results comparing intracervical and intrauterine insemination. Finally, we believe that when using intrauterine insemination with thawed sperm we also have obtained a cycle fecundity similar to that obtained using fresh semen. Our program therefore uses only quarantined, frozen semen. In your practice now do you still use fresh semen and intensive surveillance for HIV or do you use thawed semen with intrauterine insemination?

DR. ROBERT ISRAEL, Los Angeles, California. The authors have presented a superb, clear, and statistically thorough article. Most of us are using intrauterine insemination for homologous inseminations. Do you think we should go back to using the cervical cap routinely for therapeutic donor insemination, except for women with cervical factor infertility?

DR. ENRIQUE DECASTRO, Portland, Oregon. How does the thawing time affect the sperm? What is the optimum time for insemination after thawing?

DR. ADAMSON (Closing). Dr. Young focused on the difference in fecundity between fresh and frozen sperm. Our study's major strength is that it is a prospective randomized study. We do show a statistically significant difference between fresh and frozen sperm in fecundity and in 3-month cumulative pregnancy rate. These results are consistent with results reported by others in the literature after evaluation of more cycles with much less rigorous design and analysis. The difference in fecundity is especially significant for couples with multiple factors that reduce fecundity. Many couples undergo only three or four cycles of donor insemination; therefore the 3-month cumulative pregnancy rate difference is important in counseling. With fecundity of 0.094, insemination with frozen sperm will take 12 months to obtain the 68% pregnancy rate achieved in 5 months with fresh sperm. Most patients in our practice consider this a meaningful difference and prefer fresh sperm in spite of the risk of acquired immunodeficiency syndrome. This is especially true when this risk is compared with the risks of acquiring hepatitis C or dying of pregnancy complications. We perform exceedingly rigorous screening for infectious diseases to minimize the risk. We currently inform patients of the differences in the risks and benefits between fresh and frozen sperm and use our data to encourage them to undergo more cycles with frozen sperm.

The design of our study does not allow us to draw conclusions on the use of cervical cap compared with intrauterine insemination or on the effect of the number of sperm inseminated. However, our data are consistent with those reported by others that suggest that intrauterine insemination may improve pregnancy rates with frozen sperm. We agree that the total number

of motile sperm delivered is probably the most important controllable variable.

Dr. Burry, we have recently changed our approach and perform one intrauterine insemination per cycle with two "straws" of frozen sperm approximately 12 to 24 hours after a urinary LH surge. Should conception not occur after three cycles, we consider adding 150 mg of clomiphene citrate on cycle days 3 to 7 for three to six cycles and finally recommend using human menopausal gonadotropin for one to three additional cycles. For women failing to conceive after this, we recommend in vitro fertilization, adoption, or child-free living.

Dr. Israel, we use intrauterine insemination when we perform homologous insemination with controlled ovarian hyperstimulation for patients with cervical factor infertility, including poor cervical mucus, antisperm antibodies, and consistently poor postcoital or cervical mucus penetration tests. We also empirically use up to six cycles of intrauterine insemination for partners with abnormal semen analyses. For couples with sexual dysfunction alone we use cap insemination; however, when there is a scheduling difficulty we also use intrauterine insemination of homologous frozen sperm.

Dr. DeCastro, we thaw our specimens at room temperature because this method is convenient and probably as efficacious as any other. We perform the insemination immediately to maximize the number of progressively motile sperm placed in the uterus.

Effect of controlled ovarian hyperstimulation on pregnancy rates after intrauterine insemination

Sheila J. DiMarzo, PhD, Joseph F. Kennedy, MD, Philip E. Young, MD, Stephen A. Hebert, MD, Dianne C. Rosenberg, MD, and Benito Villanueva, MD
San Diego, California

OBJECTIVE: Our objective was to evaluate the effect of controlled ovarian hyperstimulation on pregnancy rates after intrauterine insemination of washed husband's sperm.
STUDY DESIGN: In a private practice setting at the Infertility, Gynecology, and Obstetrics Medical Group in San Diego, 79 treatment programs included intrauterine insemination during natural cycles, 195 included clomiphene stimulation, and 53 had human menopausal gonadotropin stimulation. Pregnancy rates and monthly fecundabilities were calculated by life-table analysis and compared by the Z test.
RESULTS: By life-table analysis the cumulative probability of pregnancy after intrauterine insemination with no ovulation induction is 21.0% after six cycles with a monthly fecundability of 3.4%. With clomiphene the pregnancy rate is 32.7% with a monthly fecundability of 6.1%, and with human menopausal gonadotropin the pregnancy rate is 60.7% with a monthly fecundability of 13.0%. The human menopausal gonadotropin group had a significantly higher pregnancy rate and monthly fecundability as compared with the other two groups. There were no differences between the no-stimulation and the clomiphene groups.
CONCLUSIONS: Human menopausal gonadotropin stimulation results in a significantly higher pregnancy rate and monthly fecundability after intrauterine insemination as compared with no stimulation or clomiphene use. (Am J Obstet Gynecol 1992;166:1607-13.)

Key words: Controlled ovarian hyperstimulation, intrauterine insemination, pregnancy rates

Intrauterine insemination with washed husband's sperm has been used as a treatment for various forms of infertility for many years in spite of the fact that questions about its effectiveness and rationale for use have abounded in the literature.[1] As techniques for sperm preparation improved,[2] largely as a result of in vitro fertilization (IVF) protocol developments, increased pregnancy rates have been reported,[3-5] whereas other reports indicate no such increases.[4, 6] Consequently, clinical use of this procedure has continued in spite of the lack of adequately controlled studies demonstrating its efficacy.

Controlled ovarian hyperstimulation followed by intrauterine insemination with husband's washed sperm has also been used with increasing frequency,[7-10] particularly in situations where a stimulation cycle fails to meet the criteria to proceed with a gamete intrafallopian transfer (GIFT) or IVF cycle.[11] Considering a patient's investment in terms of cost, time, and emotional stress it is often preferable to offer intrauterine insemination, assuming that at least one fallopian tube is patent, rather than to completely cancel the style. The success of these treatments has prompted some clinicians to recommend a trial of a few months of intrauterine insemination with controlled ovarian hyperstimulation before GIFT or IVF for patients with patent tubes.[10, 12, 13]

Within our practice patients with varying diagnoses have been offered intrauterine insemination for several years. Some received ovulation induction medications to correct ovulatory disorders and others were hyperstimulated as an empiric treatment, usually after the failure of intrauterine insemination without stimulation. This article is a retrospective study comparing pregnancy rates in three treatment groups of patients: no ovarian stimulation followed by intrauterine insemination, clomiphene treatment followed by intrauterine insemination, and human menopausal gonadotropin (hMG) hyperstimulation followed by intrauterine insemination. Individual patient couples often fell into two or all three treatment groups for one or more cycles as their workup progressed. In all cases intrauterine insemination was done with washed husband's sperm. Pregnancy rates are compared between the three treatment groups, and the patient treatment groups are described in respect to patient age, number of insem-

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Table I. Cumulative probability of pregnancy after intrauterine insemination with no ovulation induction (life-table analysis)

Cycle No.	*Observed at start of cycle*	*No. of pregnancies*	*No. lost to follow-up*	*Person months of follow-up*	*Probability of pregnancy/cycle*	*Probability of no pregnancy in cycle*	*Cumulative probability of pregnancy*
1	79	2	2	77	0.026	0.974	0.026
2	46	0	2	44	0	1	0.026
3	27	3	4	23	0.13	0.87	0.153
4	17	1	2	15	0.067	0.933	0.21
5	9	0	0	9	0	1	0.21
6	6	0	0	6	0	1	0.21
7	2	0	1	1	0	1	0.21
8	0	0	0	0	0	1	0.21

ination cycles per treatment group, number of motile sperm used for insemination, and number of inseminations per treatment cycle.

Material and methods

Couples who had undergone extensive infertility evaluations and treatments were offered intrauterine insemination of washed husband's sperm as a treatment option. They were eligible to participate if the male had any motile sperm and if the female had at least one open fallopian tube and ovulated either spontaneously or with medication. Data include all intrauterine insemination cycles with husband's washed sperm that took place within our practice in the years 1988 through 1990. If a couple in the study had insemination cycles in a prior year, those cycles were included. Many of the patient couples were evaluated for their infertility within our practice, but some were referred specifically for insemination with washed husband's sperm.

A treatment program is defined as a series of monthly cycles of intrauterine insemination with washed husband's sperm for the purpose of achieving a pregnancy. Some ovulatory cycles were natural, meaning no stimulation medications were used, some cycles were stimulated with clomiphene, and others were stimulated with hMG. Ovulation was pinpointed by home urinary luteinizing hormone (LH) testing and, occasionally, with serial ultrasonographic examinations and hormone assays measuring estradiol, progesterone, and LH (Diagnostic Products, Los Angeles). Inseminations were usually performed on the day after the LH surge when there was one insemination and on the day of the LH surge and the day after if two inseminations took place. If clomiphene was given, the dosage ranged from 25 to 200 mg/day (most commonly 50 mg/day) for 5 days in the early follicular phase. If hMG was used, injections usually began on cycle day 3 with one or two ampules and continued until day 8, at which time hormonal and ultrasonographic measurements were used to individualize the protocol to achieve the desired results. Patients on the hMG protocol received 10,000 IU of human chorionic gonadotropin when hormonal values and ultrasonographic measurements indicated mature follicles. Inseminations were timed for 36 hours later (one insemination) or 12 and 36 hours later (two inseminations). Many patients started with one treatment protocol and switched to a second or third protocol if pregnancy was not achieved. Typically, intrauterine inseminations were first done in the absence of stimulation unless specific ovulatory disorders were found. Patients not achieving pregnancy often were switched to clomiphene treatment and finally to hMG treatment in hopes of initiating a pregnancy.

Semen specimens were collected by masturbation into a sterile plastic specimen container and delivered to the laboratory within 1 hour of collection. The specimen was first analyzed for sperm count and motility with a computerized semen analysis system (CellSoft, Cryo Resources, New York) and then mixed with two to three volumes of Sperm Capacitation Medium (Irvine Scientific, Irvine, Calif). The diluted semen was centrifuged at 900 rpm for 7 minutes, the supernatant was removed, and the resulting pellet was resuspended in 2 ml of medium. After recentrifugation at 900 rpm for 4 minutes, the supernatant was removed and the pellet was resuspended to 0.3 ml with medium. A final analysis was done to determine total motile sperm for insemination, and the medium containing the washed sperm was drawn up into the transfer catheter and delivered to the examination room for insemination. The transfer catheter was a 5½-inch sterile polyethylene 3½Fr Tomcat catheter (Sherwood Medical, St. Louis) attached to a 1 ml disposable syringe. With the patient in the lithotomy position and a speculum in place, the transfer catheter containing the washed sperm was passed gently through the cervical canal and into the uterine cavity. The specimen was expelled from the catheter, and the catheter was removed. The patient rested for 10 to 15 minutes and then resumed normal activity.

Comparisons of the pregnancy rates for intrauterine insemination of husband's washed sperm in natural cy-

Table II. Cumulative probability of pregnancy after intrauterine insemination with clomiphene administration (life-table analysis)

Cycle No.	*Observed at start of cycle*	*No. of pregnancies*	*No. lost to follow-up*	*Person months of follow-up*	*Probability of pregnancy/cycle*	*Probability of no pregnancy in cycle*	*Cumulative probability of pregnancy*
1	195	12	14	181	0.066	0.934	0.066
2	127	8	4	123	0.065	0.935	0.127
3	81	8	4	77	0.104	0.896	0.218
4	49	0	2	47	0	1	0.218
5	34	1	3	31	0.032	0.968	0.243
6	19	2	1	18	0.111	0.889	0.327
7	12	0	1	11	0	1	0.327
8	7	0	0	7	0	1	0.327
9	6	0	0	6	0	1	0.327
10	4	0	0	4	0	1	0.327
11	3	0	0	3	0	1	0.327
12	2	0	0	2	0	1	0.327
13	1	0	0	1	0	1	0.327
14	0	0	0	0	0	1	0.327

cles, clomiphene-stimulated cycles, and hMG-stimulated cycles were made by the statistical methods described in detail by Cramer et al.[14] Life tables were constructed and monthly fecundabilities were determined. The Z test was used to compare the monthly fecundabilities, pregnancy rates, and the other variables discussed.

Results

For the treatment group receiving intrauterine insemination without ovulation induction, there were six pregnancies among 79 treatment programs. These 79 treatment programs included a total of 186 insemination cycles, for an average monthly fecundability of 3.2%. For the treatment group receiving intrauterine insemination after clomiphene administration, there were 31 pregnancies among 195 treatment programs. These 195 treatment programs included a total of 540 insemination cycles, for an average monthly fecundability of 5.7%. For the treatment group receiving intrauterine insemination after hMG stimulation there were 13 pregnancies among 53 treatment programs. These 53 treatment programs included a total of 104 treatment cycles, for an average monthly fecundability of 12.5%. By Z test analysis the differences in monthly fecundabilities between no ovulation induction versus hMG stimulation is significant ($p = .005$) as is that between clomiphene administration and hMG stimulation ($p = 0.02$). The monthly fecundability for intrauterine insemination with clomiphene administration is not statistically different from that after no ovulation induction.

The life-table analysis for the cumulative probability of pregnancy after intrauterine insemination for each of the treatment groups are shown in Tables I, II, and III. In Table I the cumulative probability of pregnancy following IUI with no ovulation induction is 21.0% after six insemination cycles. This life-table analysis estimates the monthly fecundability as 3.4% with a 95% confidence interval of 0.7% to 6.2%. In Table II the cumulative probability of pregnancy after intrauterine insemination and clomiphene administration is 32.7% after six insemination cycles. This life-table analysis estimates the monthly fecundability as 6.1% with a 95% confidence interval of 3.9% to 8.2%. In Table III the cumulative probability of pregnancy after intrauterine insemination after hMG stimulation is 60.7% after six insemination cycles. The life-table analysis estimates the monthly fecundability as 13.0% with a 95% confidence interval of 5.9% to 20.1%. By Z test analysis the differences in monthly fecundabilities between no ovulation induction versus hMG stimulation is significant ($p = 0.006$) as is that between clomiphene administration and hMG stimulation ($p = 0.026$). The monthly fecundability for intrauterine insemination with clomiphene administration is not statistically different from that after no ovulation induction. The differences between the cumulative probability of pregnancy after six insemination cycles for intrauterine insemination with no ovulation induction versus hMG stimulation is significant ($p < 0.001$), as is that between clomiphene administration and hMG stimulation ($p < 0.001$). The cumulative probability of pregnancy after six insemination cycles for intrauterine insemination with clomiphene administration is not statistically different from that after no ovulation induction.

To determine if there was some variable that was different among the three treatment groups which might explain these differences in pregnancy rates, several factors were investigated, as shown in Table IV. This table indicates that in terms of the age of the woman, the number of insemination cycles per treat-

Table III. Cumulative probability of pregnancy after intrauterine insemination with hMG stimulation (life-table analysis)

Cycle No.	*Observed at start of cycle*	*No. of pregnancies*	*No. lost to follow-up*	*Person months of follow-up*	*Probability of pregnancy/cycle*	*Probability of no pregnancy in cycle*	*Cumulative probability of pregnancy*
1	53	6	3	50	0.12	0.88	0.12
2	27	4	1	26	0.154	0.846	0.256
3	13	1	0	13	0.077	0.923	0.313
4	7	1	0	7	0.143	0.857	0.411
5	3	1	0	3	0.333	0.667	0.607
6	1	0	0	1	0	1	0.607
7	0	0	0	0	0	1	0.607

Table IV. Values of several factors descriptive of treatment groups

Factor	*No ovulation induction*		*Clomiphene administration*		*hMG stimulation*	
	Mean ± SD	*Range*	*Mean ± SD*	*Range*	*Mean ± SD*	*Range*
Age of female patient (yr)						
Total	33.2 ± 3.4	27-40	33.5 ± 4.3	25-46	34.4 ± 4.4	24-46
Pregnant	31.7 ± 1.9	29-34	31.7 ± 3.9	25-39	33.9 ± 2.6	28-38
Not pregnant	33.3 ± 3.4	27-40	33.6 ± 4.3	25-46	34.5 ± 4.9	24-46
No. of insemination cycles per treatment program						
Total	2.3 ± 1.6	1-7	2.8 ± 2.1	1-13	2.0 ± 1.3	1-6
Pregnant	1.8 ± 0.7	1-3	2.2 ± 1.4	1-6	2.0 ± 1.2	1-5
Not pregnant	2.3 ± 1.6	1-7	2.9 ± 2.3	1-13	2.0 ± 1.3	1-6
Motile sperm (millions) per insemination cycle						
Total	27 ± 35	1-240	41 ± 55	1-396	41 ± 44	1-194
Pregnant	40 ± 49	3-33	48 ± 73	2-396	48 ± 34	2-123
Not pregnant	27 ± 35	1-240	40 ± 55	1-387	40 ± 45	1-194
No. of inseminations per treatment cycle						
Total	1.4 ± 0.5	1-3	1.4 ± 0.5	1-4	1.7 ± 0.5	1-3
Pregnant	1.2 ± 0.4	1-2	1.4 ± 0.5	1-2	1.7 ± 0.5	1-2
Not pregnant	1.4 ± 0.5	1-3	1.4 ± 0.5	1-4	1.7 ± 0.5	1-3

ment program, the number of motile sperm per insemination cycle, and the number of inseminations per treatment cycle there were no differences between the three treatment groups. In addition, the percent of patients in each treatment group who had never been pregnant (before starting the treatment program) was not different (51% for intrauterine insemination without ovulation induction, 51% for intrauterine insemination after clomiphene administration, and 46% for intrauterine insemination after hMG stimulation). Table IV also shows that there is no difference in any of these variables between the pregnant and nonpregnant patients within each of the three treatment groups.

Each treatment group consisted of a very diverse patient population. Some patient couples had idiopathic infertility, whereas others had one or more male and/or female infertility factors present. The distribution of the numbers of male or female infertility factors did not differ between the treatment groups nor did it differ between the pregnant and nonpregnant patients within each group.

The treatment group that had intrauterine insemination after hMG stimulation had the highest overall pregnancy rate, although many of these patients (38%) had undergone an average of 4.6 ± 3.1 (range 1 to 11) insemination cycles with no ovulation induction or clomiphene administration before hMG stimulation. Of the hMG-stimulated patients who achieved a pregnancy, 54% had undergone an average of 3.6 ± 3.5 (range 1 to 11) insemination cycles in one or both of the other two treatment groups.

Comment

This retrospective study of patient couples receiving intrauterine insemination treatment with husband's washed sperm clearly demonstrates a significantly increased pregnancy rate and monthly fecundability after controlled ovarian hyperstimulation with hMG as com-

pared with the pregnancy rates in the absence of ovulation induction or with the use of clomiphene. These differences are not due to differences in the patient populations because they are very similar in terms of the age of the female patient, her gravidity, the number of infertility factors, the number of intrauterine insemination treatment cycles, the number of motile sperm per intrauterine insemination cycle, and the number of inseminations per intrauterine insemination cycle. If anything, the study design should have skewed the results toward lower pregnancy rates with intrauterine insemination after hMG stimulation because a large number of these patient couples had already failed with intrauterine insemination in the absence of ovulation induction or with clomiphene administration. It is of interest to note that clomiphene use did not increase the pregnancy rate after intrauterine insemination in this study, a finding supported by Martinez et al.[15]

In our clinical practice we continue to offer intrauterine insemination with washed sperm for treatment in patients with a variety of infertility factors. In some cases the rationale is straightforward, such as with oligospermia or cervical factor. In other cases, such as ovulation disorders or endometriosis, the treatment is more empiric. The addition of controlled ovarian hyperstimulation to the intrauterine insemination protocol is clearly therapeutic in the presence of ovulatory disorders and empiric in most others. This study supports our clinical approach and, in fact, has led us to consider hMG stimulation sooner in a patient's treatment, especially after four to six failed intrauterine insemination cycles with clomiphene administration. This clinical approach and the finding of a limited success after four to six treatment cycles is supported in the literature.[11, 16, 17]

Drawbacks to the use of hMG stimulation before intrauterine insemination include the relative cost of a treatment cycle in view of the medication costs, the necessity of increased monitoring by hormone assays and ultrasonographic measurements, and the risks of ovarian hyperstimulation and multiple gestation. Although the cost of an hMG-stimulated intrauterine insemination cycle is expensive, it is still substantially less expensive than an IVF or GIFT procedure. The medical risks of hMG stimulation can be minimized by very careful preovulatory monitoring of the changes in follicle size and number and the increase in hormonal levels. Consequently, hMG stimulation followed by intrauterine insemination appears to be a reasonable treatment approach before IVF or GIFT treatment for many patients.[11, 12]

Recent literature reviews have carefully examined the rationale and success of this treatment approach.[13, 18] Explanations for a higher pregnancy rate for empiric hMG therapy and intrauterine insemination include the opportunity for increased gamete density at the site of fertilization and the potential correction of subtle ovulatory disorders.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

DR. KENNETH A. BURRY, Portland, Oregon. Dr. Villanueva and his colleagues have retrospectively reviewed their clinical experience of 253 treatment programs from 1988 through 1990 in which intrauterine insemination was performed. Their conclusion is that performing this procedure after hMG stimulation yields a significantly higher pregnancy rate, or monthly fecundability, as compared with intrauterine insemination after no ovulation induction or clomiphene treatment.

They divided the treatment programs into three groups: (1) intrauterine insemination in a spontaneously ovulatory cycle, (2) intrauterine insemination after clomiphene citrate stimulation, and (3) intrauterine insemination after hMG stimulation. These empiric treatment regimens were used for multiple infertility factors, both male and female. Many of the couples themselves had multiple factors contributing to their infertility.

Intrauterine insemination with and without a male factor has also been used empirically for the treatment of multiple factors causing infertility. The literature gives us a mixed message concerning the efficacy of this therapy. Kerin et al.[1] claim that intrauterine insemination was superior to coitus in couples with impaired semen quality. Cruz et al.[2] found it superior to intrauterine intracervical insemination in male factor infertility. On the other hand, te Velde et al.[3] showed that intrauterine insemination was no better than coitus for male factor; however, it was efficacious in women with cervical factor. A larger study by Kirby et al.[4] of 285 couples compared 600 cycles of intrauterine insemination with 505 cycles of coitus timed by an LH surge. They found no benefit to intrauterine insemination except for severe male factor.

GIFT and IVF are currently used as a last resort by many couples with long-standing infertility. These are expensive technologies with relatively low probability of pregnancy. Welner et al.[5] reported a significant pregnancy rate in 97 couples who received hMG before proceeding to IVF. Many centers are currently offering ovarian hyperstimulation and intrauterine insemination as an option before these assisted reproductive technologies.

Empiric ovulation induction in ovulatory women has been used the treatment of unexplained infertility or conditions associated with low fecundability, such as endometriosis. Deaton et al.[6] reported an increase in cycle fecundity in women treated with clomiphene and intrauterine insemination. Martinez et al.[7] however, saw an increased pregnancy rate with intrauterine insemination only and not with clomiphene. The experience with hMG and intrauterine insemination is more consistent. Dodson et al.[8] reported their experience with hMG and intrauterine insemination in 1987. They showed cycle fecundities of 0.17 for endometriosis, 0.29 for cervical factor, and 0.19 for idiopathic infertility. The editorial comment at the end of their article is worth repeating: "The decision to publish this controversial manuscript has been made with the intent of stimulating debate. The referees feel strongly that, before advocating intrauterine insemination during hMG-stimulated cycles, a prospective controlled study with critically evaluated infertile patients is mandatory. The advocating of this essentially empiric therapy cannot be supported by the rather meager retrospective data presented. The authors agree that they cannot state that this approach is effective therapy but have reported their observed cycle fecundity with its use. Clinicians should be discouraged from applying this therapy until controlled prospective studies can support this approach." This year, Chaffkin et al.[9] reported on 322 couples observed for 751 cycles. They found that hMG and IUI were more efficacious than either modality alone. Although their study reviewed more patients than previous studies, this was another retrospective evaluation of clinical experience. Dodson and Haney[10] reviewed the literature and reported in *Fertility and Sterility*. Their concluding sentence reads, "When the relatively low direct and indirect costs of controlled ovarian hyperstimulation and IUI [intrauterine insemination] are considered, acknowledging the lack of prospective, controlled studies, this procedure appears to be at least as cost-effective as GIFT and IVF."[10]

The conclusions by Dr. Villanueva are consistent with the majority of the literature on this subject. The weakness in the article is that it is another retrospective evaluation of clinical experience. Another concern in the interpretation of their results is the multiple etiologies for infertility in their patient population.

Endometriosis is associated with low fecundability. Ovulatory disorders are associated with a near-normal fecundability when appropriately treated. Male factor infertility is a broad category, and the number of normal motile cells available for insemination is important. Therefore variations in patient populations between groups within a study, as well as between studies, may lead to a significant bias of results.

I have these questions for Dr. Villanueva: (1) You state there are no differences between the three groups. However, I see that 62.3% of the no-ovulation induction group had endometriosis compared with 40.3% of the clomiphene group and 22% of the hMG group. Do you think endometriosis has an impact on the differences in pregnancies among these three groups? (2) Similarly,

the no-induction group had 41.6% of couples with normal male factor compared with the clomiphene group with 59.7% and the hMG group with 66% normal male factor. Was this not significant? (3) On the basis of your experience, what are your current recommendations for management of unexplained infertility or endometriosis in patients with long-standing infertility?

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Dr. Simon Henderson, San Francisco. To do intrauterine insemination or not to do it, that is the question. This article does not really answer the question, a question that begs to be answered. Dr. Villanueva, can you tell us your pregnancy rates for clomiphene and gonadotropin treatment without intrauterine insemination? This information would help us approach an answer to the question of whether intrauterine insemination is a necessary part of the treatment.

Dr. Miles Novy, Portland, Oregon. I echo Dr. Burry's comment about the need for prospective controlled studies. It is imperative that in evaluating the efficacy of these methods we compare fairly large groups that have comparable causes of infertility. Dr. Villanueva, an hMG–intrauterine insemination cycle can cost ≥$2000 by the time you add up the costs of the drug and the estradiol and ultrasonographic monitoring. The objective of treatment in these women is to prevent severe ovarian hyperstimulation and to obtain a limited number of follicles, maybe two or three. This objective is quite different from that with hyperstimulation for IVF. This milder effect is frequently achieved with 100 or 150 mg of clomiphene per day and a simple monitoring regimen with a total cost of a few hundred dollars. Would you comment on costs? Finally, Dr. Villanueva, please tell us more about the idiopathic infertility group. It seemed to me from your presentation that there were only 11 patients with idiopathic infertility, that only two were in the hMG group and neither became pregnant.

Dr. Donald Smith, Seattle, Washington. Did you use progesterone supplementation in the luteal phase for your hMG patients?

Dr. Villanueva (Closing). Dr. Burry, thank you for your evaluation. I repeat that we found no statistical difference in the probability of pregnancy between these groups irrespective of their infertility cause. Also, in the no-stimulation group, a group that had a higher percentage of patients with endometriosis, there was also a higher percentage of pregnancies in patients with that diagnosis. The difference was not statistically significant. We now first offer our patients with long-standing infertility intrauterine insemination without ovulation induction. We still use clomiphene stimulation empirically for a short time, perhaps four or six cycles. Then, rather than continuing or moving on to the admittedly more expensive and complicated IVF or GIFT, we offer hMG stimulation with intrauterine insemination.

Dr. Henderson asked whether we have data on cycles with hMG clomiphene stimulation without intrauterine insemination. Unfortunately, I have none at this time.

Dr. Novy, we are very mindful of the cost. I agree with you and with Dr. Burry about the need for a prospective study. Until this is done, these questions will be open to argument.

Dr. Smith, we do not use progesterone supplements.

Percutaneous umbilical blood sampling: Results from a multicenter collaborative registry

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Percutaneous umbilical blood sampling has become an important diagnostic tool in the evaluation of fetal health. Although recent reports have established the safety of this procedure, most have arisen from single centers with extensive experience. The Western Perinatal Collaborative Group has maintained a continuous registry of percutaneous umbilical blood sampling performed at the 13 member institutions for the years 1986 through 1990. During this period 302 procedures were performed. Primary indications for percutaneous umbilical blood sampling included Rh and non-Rh isoimmunization, intrauterine growth retardation, nonimmune hydrops, fetal anomalies, immune thrombocytopenia purpura, and rapid fetal chromosome analysis. Percutaneous umbilical blood sampling was successfully performed in 93.7% of cases with a sample withdrawn by a single puncture in 80.5% of attempts. In the majority of cases a 22-gauge needle was used. Postprocedural fetal death resulted in six cases (2.1%) and was most often associated with fetal anomalies or an abnormal karyotype. In conclusion, evaluation of the fetal state by percutaneous umbilical blood sampling is a safe and easily performed procedure, as demonstrated by a collaborative multicenter study. (Am J Obstet Gynecol 1992;166:1614-8.)

Key words: Percutaneous umbilical blood sampling, fetal anomalies, Rh isoimmunization

Percutaneous umbilical blood sampling has become an increasingly more important and frequently performed diagnostic procedure in evaluating fetal health.[1-3] This relatively new technology has also allowed more direct access to the fetus for treatment in conditions such as Rh isoimmunization.[4]

Although fetal bradycardia and uterine irritability are recognized transient side effects of percutaneous umbilical blood sampling,[5] serious complications such as fetal death appear to be quite uncommon. The safety of the procedure, however, has largely been established from studies originating from single centers with extensive experience of only a few operators.

As tertiary level perinatal centers in the United States have greatly increased over the last decade, invasive obstetric procedures are now performed at a large number of locations, quite in contrast to the more centralized experience reported in other countries.[6-8] We conducted a multicenter investigation to assess the safety of the procedure.

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Material and methods

The Western Perinatal Collaborative Group is a nonprofit research organization that maintains a registry of invasive obstetric procedures performed at its 13 member institutions. Each institution performed percutaneous umbilical blood sampling by its own method, and a protocol for the procedure was neither developed nor suggested. During the time period of 1986 through 1990, a total of 302 procedures were attempted for diagnostic purposes at six of the 13 institutions. Data were collected on standardized forms and included information regarding indications for the procedure, technical details, ultrasonographic findings, tests performed, procedure-related complications, and perinatal outcome. For the purpose of common reporting, we used 3 days after the procedure as the definition of an intrauterine death. Statistical analysis was performed with the Statistical Program for the Social Sciences.

Results

During the study period, 302 percutaneous umbilical blood sampling procedures were attempted between 16 and 39 gestational weeks for a variety of indications (Table I). The most frequent reasons included Rh isoimmunization, idiopathic thrombocytopenia, nonimmune hydrops, fetal anomalies, and idiopathic thrombocytopenia purpura. Of the 302 procedures, 283 were accomplished, for a success rate of 93.7%.

The procedure was performed most frequently with a 22-gauge needle (81.6% of cases) and next most commonly with a 20-gauge needle (15.6% of cases). A 25-gauge needle was used in <3% cases (Table II). The placenta was located anteriorly in 65.5% of cases and posteriorly in 30.2%. In <5% of percutaneous umbilical blood sampling was the placenta fundal or a previa. Cord sampling was performed by an approach next to the placental insertion site in 89.6% and adjacent to the fetus or in a free loop in approximately 10% of cases. Antibiotic treatment of the mother was not routinely used at any center. During or after the procedure tocolytic agents were used in 33.0% of cases.

Immediate procedural complications occurred infrequently, with only a single case leading to immediate delivery due to a prolonged bradycardia that, at the time of cesarean section, was found to be caused by an inadvertent cord laceration. Transient streaming of blood from the cord was common (89.4%), but it persisted for longer than 1 minute in only 10.6% of cases (Table III). Fetal heart rate changes were infrequent, occurring <7% of the time.

More severe complications leading to fetal death within 3 days of the procedure occurred in six cases (2.1%). Four of the six cases were performed for fetal anomalies and the remaining two for severe Rh isoimmunization (Table IV). In one case of multiple anomalies, a karyotype of trisomy 18 returned after fetal death had occurred.

Table I. Primary indication for percutaneous umbilical blood sampling (N = 302)

Indication	*No.*
Rh isoimmunization	65
Intrauterine growth retardation	45
Non-Rh isoimmunization	44
Nonimmune hydrops	31
Multiple anomalies	22
Neural tube defects	21
Idiopathic thrombocytopenic purpura	20
Abnormal amniocentesis	12
Other*	42

*Includes (<10 each) cardiac malformations, congenital infections, pleural effusions, fetal distress, alloimmune thrombocytopenia, hemoglobinopathies, metabolic disorder risks.

Table II. Procedural characteristics (n = 283)

Characteristic	%
Needle size	
20 gauge	15.6
22 gauge	81.6
25 gauge	2.8
Placental location	
Anterior	65.5
Posterior	30.2
Fundal	4.0
Previa	0.4
Location of insertion	
Placental	89.8
Fetal	4.3
Free loop	5.9
Use of tocolytics	
Yes	33.0
No	67.0

Comment

Our results from a multicenter collaborative registry support the safety and effectiveness of percutaneous umbilical blood sampling. The indications for the procedure in this collaborative registry are similar to those reported in the literature, with the exception of Rh and nonisoimmunization being two of the three most common primary indications.[1, 2] This may reflect differences in the referral patterns and a relatively more aggressive approach to the patient with a high titer indirect Coombs' test of institutions participating in this study. The direct determination of fetal disease severity through hematologic (rather than amniotic fluid testing) and the potential elimination of serial amniocentesis in mothers with an antigen-negative fetus are important advantages of percutaneous umbilical blood sampling. Forty-five procedures were done for severe intrauterine growth retardation. This is an important indication for percutaneous umbilical blood sampling, as 17% of severely growth retarded fetuses were found to have chromosomal anomalies in a recent series by Bilardo and Nicolaides.[9]

The majority of cases in this study were performed with a 22-gauge needle, which is similar in size to that described by others.[8, 10] In one of the largest series to date, however, Daffos et al.[6] universally used a 20-gauge needle with a very low reported complication rate.[6] Few data exist relating procedural complications to needle size, and to our knowledge, no randomized trials addressing this question have been performed.

An anterior placental location site was noted in 65.5% of our cases, which is in agreement with results reported by Orlandi et al.[7] The investigators in our series obtained samples from the cord adjacent to the placenta in 89.9% of cases and from a free loop or near the fetus in the remaining 10.2% of cases. Sampling sites were at the placental cord origin in 63.5% of 96 cases reported by Weiner[10] and 72.2% of 322 cases described by Boulot et al.[8]

The duration of blood streaming from the cord and fetal heart rate changes after sampling have been examined as potential markers for procedural complications. We found bleeding for longer than 1 minute from the cord puncture site in 10.6% of cases. In one case an immediate cesarean section was performed

Table III. Procedural complications (n = 283)

	%
Duration of streaming	
<1 min	89.4
≥1 min	10.6
Fetal heart rate change	
None	93.5
Bradycardia	5.8
Tachycardia	0.7

Table IV. Fetal death within 3 days of procedure

Case No.	*Indication*	*Gestational age (wk)*	*Karyotype*
1	Encephalocele	22	ND
2	Nonimmune hydrops	30	Normal
3	Multiple anomalies, fetal distress	23	ND
4	Multiple anomalies	27	47 ± 18
5	Rh isoimmunization	32	ND
6	Rh isoimmunization	24	ND

ND, Not done.

where a small cord laceration was found. No apparent adverse neonatal outcome resulted. This figure is well within the range of 6% to 13% described by others.[6, 11] Our 5.8% rate of transient bradycardia after percutaneous umbilical blood sampling was also similar to the 3% to 12% described by others.[6, 10, 11] Although cord hematomas and amnionitis have been described in other series, we did not encounter either of these complications.

Unexpected fetal death after a percutaneous umbilical blood sampling procedure is clearly the most serious of all potential complications. This risk may be modified by a number of factors including the severity of fetal disease at the time of the procedure, the intrauterine natural history of the disease for which the fetus is being tested, the gestational age at the time, and the experience of the operator. Intrauterine death rates may be expected to vary from center to center, depending on the above considerations. For example, the fetus with hydrops or an autosomal trisomy has a poor intrauterine prognosis even without the potential added risk associated with the diagnostic procedure. In addition, gestational age at the time of testing may modify the direct risk of the procedure[7] and limit therapeutic options. For instance, a fetus of advanced gestational age may be delivered after a procedural complication and treated in the nursery. In contrast, the option of delivering a very immature fetus would not exist and an intrauterine death may be more likely.

Although operator experience is undoubtedly important with respect to the safety and effectiveness of diagnostic procedures such as percutaneous umbilical blood sampling, it has been argued that it should only be performed at large centers with extensive and continuing experience.[8] Reports addressing this question, however, have been mixed. Boulot et al.[8] found a rate of fetal death in utero of 1.9% in a report of 322 percutaneous umbilical blood samplings, performed in 277 pregnancies. All complications occurred in the first 30 procedures performed by each operator and none in the subsequent 210 procedures. In a study of 500 percutaneous umbilical blood sampling procedures performed between 12 and 21 weeks by a single operator, Orlandi et al.,[7] to the contrary, found no relationship between experience and the rate of fetal loss. As many fetal deaths were encountered in the first 100 procedures as in the last 100 in the series.

Our intrauterine fetal death rate of 2.1% compares favorably with reports of others.[6-8] With the low frequency of fetal death and clinically and demographically dissimilar populations, statistical comparison of our study with others would not be meaningful. Four of the intrauterine deaths in our series were associated with fetal anomalies or chromosomal aneuploidies. The remaining two fetal deaths were complicated by Rh isoimmunization at 24 and 32 weeks. No immediate complications were noted in either Rh isoimmunization case and causes for intrauterine deaths were not apparent. It could be argued, however, that the 32-week gestation complicated by Rh isoimmunization could potentially have survived had the complication been immediately recognized.

In conclusion, the results from this multicenter study of 283 percutaneous umbilical blood sampling procedures performed at six institutions compare favorably with reports from other centers with respect to procedure-related complications and intrauterine deaths. Percutaneous umbilical blood sampling is a safe and effective procedure that offers wide clinical application for disease diagnosis and treatment in the fetus.

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Discussion

DR. RUSSELL K. LAROS, JR., San Francisco, California. Percutaneous umbilical blood sampling has become an important part of our diagnostic and therapeutic armamentarium. Issues currently being debated include indications, risks, and who should be performing the procedure. Because of concerns about misuse, the California State Department of Health is currently promulgating rules and regulation aimed at controlling how, why, and where it will be done. Although the state's motivation is patient protection, I believe we should resist this degree of governmental control of medical practice. The data presented by Dr. Hickok and his colleagues are important in that they address the issues of safety and indications in a multiinstitution setting.

Dr. Hickok has told us about 302 percutaneous umbilical blood sampling procedure attempted at six institutions, thus providing us with one of the largest collective experiences reported to date. He has carefully described indications, methodology, and outcome. I do have six comments or questions that should further clarify his data. (1) Your abstract and Material and methods section imply that 13 institutions contributed cases to the study. It appears to me that only six of the 13 really contributed. Is this correct? (2) How many operators were actually performing percutaneous umbilical blood sampling? Because of the intense interest in the issue of the relationship between operator experience and outcome, please tell us about the frequency of fetal loss and failed procedures as related to operator experience. (3) My arithmetic indicates that the six fetal losses all occurred in cases of successful percutaneous umbilical blood sampling (i.e., 6/283); is this a correct interpretation? If so, the lack of mortality in the unsuccessful cases is reassuring as we all worry about the fetal consequences of multiple, unsuccessful "sticks." (4) Could you explain the reasons for percutaneous umbilical blood sampling rather than amniocentesis in the 21 cases of neural tube defects? Were these cases too far along in gestation to get the same data with the safer procedure? (5) Dr. Hickok is aware of our approach to the delivery of patients with idiopathic thrombocytopenic purpura. Did the information obtained in the 20 cases of idiopathic thrombocytopenic purpura influence the route of delivery in any case? and (6) Finally, in your opinion, how many percutaneous umbilical blood sampling procedures per year per operator are required to maintain proficiency?

I congratulate Dr. Hickok and associates for reporting this important experience. The issue of operator proficiency is especially critical as we consider how to train perinatologists in this technology.

DR. JULIAN T. PARER, San Francisco, California. Dr. Hickok, can you call a procedure with a mortality of 2% a safe procedure? In a day when the overall perinatal mortality is <2%, I believe this is a high rate of mortality. Acknowledging that not all of these deaths are due to the procedure, I would conclude that we should reserve cordocentesis for grave indications and I think you probably agree. We cannot agree on some of your indications, however. One is the use of cordocentesis for early diagnosis of fetal blood type in cases of red cell isoimmunization. It seems to me that because amniocentesis has a mortality of almost zero, and cordocentesis is between 1% and 2%, it is better to use serial amniocenteses as an initial approach to rule in or out severe isoimmunization, rather than to expose the fetus to the risk of cordocentesis.

DR. JACK R. LAMEY, Seattle, Washington. Dr. Hickok, I agree that it is very important for the operator to be very experienced. However, another point that I think is very important was not brought out. If the ultrasonographer is not capable and experienced, the operator is placed at a marked disadvantage.

DR. PETER WATSON, Portland, Oregon. As a participant in the collection of the data, I know, Dr. Hickok, that you have done a tremendous amount of work. Your presentation indicates that you have given this matter a great deal of thought. In answering Dr. Laros' comment about training and experience, please explain how would you advise an obstetrician who would like to perform percutaneous umbilical blood sampling for the first time. How should this person prepare for a procedure that, as noted by Dr. Parer, carries a substantial risk?

DR. DUNCAN NEILSON, Portland, Oregon. My question relates to the long term follow-up of the fetus. In the fetus the foramen ovale is open, creating the possibility of embolization to the brain from procedures done on the peripheral circulation. Injuries from that source may not become apparent until some months downstream. In your study or in other large scale collaborative studies do you look for subtle injury of that type at follow-up?

DR. LOUIS A. VONTVER, Seattle, Washington. I am puzzled by the use of this test for idiopathic thrombocytopenic purpura. If the baby has a coagulation disorder, percutaneous umbilical blood sampling is a much more dangerous test. If the baby doesn't have it, the test doesn't need to be done. Why recommend per-

cutaneous umbilical blood sampling for this purpose? Do you feel that there is no increased danger?

Dr. Hickok (Closing). Dr. Laros, it is with trepidation that I begin this discussion with you seated there. It recalls a scene 18 years ago when I was a third-year medical student and you were the junior faculty member. It is true that there were only six institutions of the 13 that collected all of the information that we needed. For the others, the data was incomplete or missing. I don't have exact information about how many operators performed percutaneous umbilical blood sampling at each center, but it was generally two or three.

The use of percutaneous umbilical blood sampling allows us to obtain a fetal karyotype quickly in situations where a fetal anomaly is discovered at an advanced gestational age. The karyotype from cultured peripheral leukocytes in the fetal circulation can be obtained in 48 hours. With amniocentesis and culture of amniocytes, it takes 7 to 14 days. Because of the increased chance of an abnormal karyotype in the fetus with a neural tube defect, parents were offered a choice of this potentially more risky procedure to have the benefits of a rapid karyotype.

Both you and Dr. Vontver asked why this approach was used in patients with idiopathic thrombocytopenic purpura. I have not looked in detail for what information was obtained in these 20 cases and how it was used. Perhaps I will join into that argument next year at this meeting.

Finally, you and others asked how many percutaneous umbilical blood sampling procedures per year per operator are required to maintain proficiency? It is not easy to answer this very important question. By and large the same operators that do percutaneous umbilical blood sampling do multiple other ultrasonographically guided procedures requiring hand-eye coordination, amniocentesis, chorionic villus sampling, and fetal tissue biopsy. It is difficult to separate percutaneous umbilical blood sampling from these other diagnostic procedures done by perinatologists in terms of the training required to attain proficiency or the amount of practice needed to maintain proficiency.

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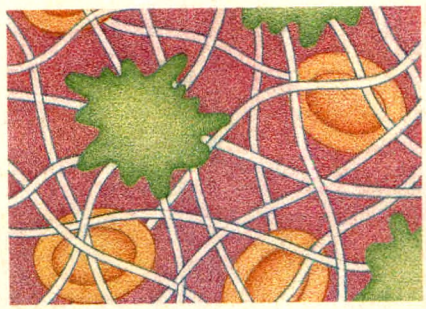
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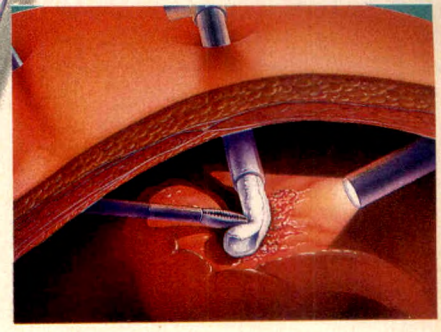
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* Management of eclampsia
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Indications and Usage

Oral contraceptives are indicated for the prevention of pregnancy in women who elect to use this product as a method of contraception.

Contraindications

Oral contraceptives should not be used in women with any of the following conditions: thrombophlebitis or thromboembolic disorders, a past history of deep-vein thrombophlebitis or thromboembolic disorders, cerebral-vascular or coronary-artery disease, known or suspected carcinoma of the breast, carcinoma of the endometrium or other known or suspected estrogen-dependant neoplasia, undiagnosed abnormal genital bleeding, cholestatic jaundice of pregnancy or jaundice with prior pill use, hepatic adenomas or carcinomas, known or suspected pregnancy.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral-contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risks of several serious conditions including myocardial infarction, thromboembolism, stroke, hepatic neoplasia, gallbladder disease, and hypertension, although the risk of serious morbidity or mortality is very small in healthy women without underlying risk factors. The risk of morbidity and mortality increases significantly in the presence of other underlying risk factors such as hypertension, hyperlipidemias, obesity and diabetes. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks. The information contained in this package insert is principally based on studies carried out in patients who used oral contraceptives with higher formulations of estrogens and progestogens than those in common use today. The effect of long-term use of the oral contraceptives with lower formulations of both estrogens and progestogens remains to be determined. Throughout this labeling, epidemiological studies reported are of two types: retrospective or case control studies and prospective or cohort studies. Case control studies provide a measure of the relative risk of disease, namely, a ratio of the incidence of a disease among oral-contraceptive users to that among nonusers. The relative risk does not provide information on the actual clinical occurrence of a disease. Cohort studies provide a measure of attributable risk, which is the difference in the incidence of disease between oral-contraceptive users and nonusers. The attributable risk does provide information about the actual occurrence of adisease in the population. For further information, the reader is referred to a text on epidemiological methods.

1. *Thromboembolic Disorders and Other Vascular Problems*

a. *Myocardial Infarction.* An increased risk of myocardial infarction has been attributed to oral-contraceptive use. This risk is primarily in smokers or women with other underlying risk factors for coronary-artery disease such as hypertension, hypercholesterolemia, morbid obesity, and diabetes. The relative risk of heart attack for current oral-contraceptive users has been estimated to be two to six. The risk is very low under the age of 30. Smoking in combination with oral-contraceptive use has been shown to contribute substantially to the incidence of myocardial infarctions in women in their mid-thirties or older with smoking accounting for the majority of excess cases. Mortality rates associated with circulatory disease have been shown to increase substantially in smokers over the age of 35 and non-smokers over the age of 40 among women who use oral contraceptives. Oral contraceptives may compound the effects of well-known risk factors, such as hypertension, diabetes, hyperlipidemias, age and obesity. In particular, some progestogens are known to decrease HDL cholesterol and cause glucose intolerance, while estrogens may create a state of hyperinsulinism. Oral contraceptives have been shown to increase blood pressure among users (see section 9 in "Warnings"). Similar effects on risk factors have been associated with an increased risk of heart disease. Oral contraceptives must be used with caution in women with cardiovascular disease risk factors.

b. *Thromboembolism.* An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Case control studies have found the relative risk of users compared to nonusers to be 3 for the first episode of superficial venous thrombosis, 4 to 11 for deep-vein thrombosis or pulmonary embolism, and 1.5 to 6 for women with predisposing conditions for venous thromboembolic disease. Cohort studies have shown the relative risk to be somewhat lower, about 3 for new cases and about 4.5 for new cases requiring hospitalization. The risk of thromboembolic disease due to oral contraceptives is not related to length of use and disappears after pill use is stopped. A two-to four-fold increase in relative risk of postoperative thromboembolic complications has been reported with the use of oral contraceptives. The relative risk of venous thrombosis in women who have predisposing conditions is twice that of women without such medical conditions. If feasible, oral contraceptives should be discontinued at least four weeks prior to and for two weeks after elective surgery of a type associated with an increase in risk of thromboembolism and during and following prolonged immobilization. Since the immediate post partum period is also associated with an increased risk of thromboembolism, oral contraceptives should be started no earlier than four to six weeks after delivery in women who elect not to breast-feed, or a midtrimester pregnancy termination.

c. *Cerebrovascular diseases.* Oral contraceptives have been shown to increase both the relative and attributable risks of cerebrovascular events (thrombotic and hemorrhagic strokes), although, in general, the risk is greatest among older (>35 years), hypertensive women who also smoke. Hypertension was found to be a risk factor for both users and nonusers, for both types of strokes, while smoking interacted to increase the risk for hemorrhagic strokes. In a large study, the relative risk of thrombotic strokes has been shown to range from 3 for normotensive users to 14 for users with severe hypertension. The relative risk of hemorrhagic stroke is reported to be 1.2 for nonsmokers who used oral contraceptives, 2.6 for smokers who did not use oral contraceptives, 7.6 for smokers who used oral contraceptives, 1.8 for normotensive users and 25.7 for users with severe hypertension. The attributable risk is also greater in older women.

d. *Dose-related risk of vascular disease from oral contraceptives.* A positive association has been observed between the amount of estrogen and progestogen in oral contraceptives and the risk of vascular disease. A decline in serum high-density lipoproteins (HDL) has been reported with many progestational agents. A decline in serum high-density lipoprateins has been associated with an increased incidence of ischemic heart disease. Because estrogens increase HDL cholesterol, the net effect of an oral contraceptive depends on a balance achieved between doses of estrogen and progestogen and the nature and absolute amount of progestogen used in the contraceptive. The amount of both hormones should be considered in the choice of an oral contraceptive. Minimizing exposure to estrogen and progestogen is in keeping with good principles of therapeutics. For any particular estrogen/progestogen combination, the dosage regimen prescribed should be one which contains the least amount of estrogen and progestogen that is compatible with a low failure rate and the needs of the individual patient. New acceptors of oral-contraceptive agents should be started on preparations containing less than 50 mcg of estrogen.

e. *Persistence of risk of vascular disease.* There are two studies which have shown persistence of risk of vascular disease for ever-users of oral contraceptives. In a study in the United States, the risk of developing myocardial infarction after discontinuing oral contraceptives persists for at least 9 years for women 40-49 years who had used oral contraceptives for five or more years, but this increased risk was not demonstrated in other age groups. In another study in Great Britain, the risk of developing cerebrovascular disease persisted for at least 6 years after discontinuation of oral contraceptives, although excess risk was very small. However, both studies were performed with oral-contraceptive formulations containing 50 micrograms or higher of estrogens.

2. *Estimates of Mortality from Contraceptive Use.* One study gathered data from a variety of sources which have estimated the mortality rate associated with different methods of contraception at different ages. These estimates include the combined risk of death associated with contraceptive methods plus the risk attributable to pregnancy in the event of method failure. Each method of contraception has its specific benefits and risks. The study concluded that with the exception of oral-contraceptive users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of birth control is less than that associated with childbirth. The observation of a possible increase in risk of mortality with age for oral-contraceptive users is based on data gathered in the 1970's–but not reported until 1983. However, current clinical practice involves the use of lower estrogen dose formulations combined with careful restriction of oral-contraceptive use to women who do not have the various risk factors listed in this labeling. Because of these changes in practice and, also, because of some limited new data which suggest that the risk of cardiovascular disease with the use of oral contraceptives may now be less than previously observed, the Fertility and Maternal Health Drugs Advisory Committee was asked to review the topic in 1989. The Committee concluded that although cardiovascular-disease risks may be increased with oral-contraceptive use after age 40 in healthy nonsmoking women (even with the newer low-dose formulations), there are greater potential health risks associated with pregnancy in older women and with the alternative surgical and medical procedures which may be necessary if such women do not have access to effective and acceptable means of contraception. Therefore, the Committee recommended that the benefits of oral-contraceptive use by healthy nonsmoking women over 40 may outweigh the possible risks. Of course, older women, as all women who take oral contraceptives, should take the lowest possible dose formulation that is effective.

3. *Carcinoma of the Reproductive Organs.* Numerous epidemiological studies have been performed on the incidence of breast, endometrial, ovarian and cervical cancer in women using oral contraceptives. The overwhelming evidence in the literature suggests that use of oral contraceptives is not associated with an increase in the risk of developing breast cancer, regardless of the age and parity of first use or with most of the marketed brands and doses. The Cancer and Steroid Hormone (CASH) study also showed no latent effect on the risk of breast cancer for at least a decade following long-term use. A few studies have shown a slightly increased relative risk of developing breast cancer, although the methodology of these studies, which included differences in examination of users and nonusers and differences in age at start of use, has been questioned. Some studies suggest that oral-contraceptive use has been associated with an increase in the risk of cervical intraepithelial neoplasia in some populations of women. However, there continues to be controversy about the extent to which such findings may be due to differences in sexual behavior and other factors. In spite of many studies of the relationship between oral-contraceptive use and breast and cervical cancers, a cause-and-effect relationship has not been established.

4. *Hepatic Neoplasia.* Benign hepatic adenomas are associated with oral-contraceptive use, although the incidence of benign tumors is rare in the United States. Indirect calculations have estimated the attributable risk to be in the range of 3.3 cases/100,000 for users, a risk that increases after four or more years of use. Rupture of rare, benign, hepatic adenomas may cause death through intra-abdominal hemorrhage. Studies from Britain have shown an increased risk of developing hepatocellular carcinoma in long-term (>8 years) oral-contraceptive users. However, these cancers are extremely rare in the U.S. and the attributable risk (the excess incidence) of liver cancers in oral-contraceptive users approaches less than one per million users.

5. *Ocular Lesions.* There have been clinical case reports of retinal thrombosis associated with the use of oral contraceptives. Oral contraceptives should be discontinued if there is unexplained partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions. Appropriate diagnostic and therapeutic measures should be undertaken immediately.

6. *Oral-Contraceptive Use Before or During Early Pregnancy.* Extensive epidemiological studies have revealed no increased risk of birth defects in women who have used oral contraceptives prior to pregnancy. Studies also do not suggest a teratogenic effect, particularly insofar as cardiac anomalies and limb-reduction defects are concerned, when taken inadvertently during early pregnancy. The administration of oral contraceptives to induce withdrawal bleeding should not be used as a test for pregnancy. Oral contraceptives should not be used during pregnancy to treat threatened or habitual abortion. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing oral-contraceptive use. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period. Oral-contraceptive use should be discontinued if pregnancy is confirmed.

7. *Gallbladder Disease.* Earlier studies have reported an increased lifetime relative risk of gallbladder surgery in users of oral contraceptives and estrogens. More recent studies, however, have shown that the relative risk of developing gallbladder disease among oral-contraceptive users may be minimal. The recent findings of minimal risk may be related to the use of oral-contraceptive formulations containing lower hormonal doses of estrogens and progestogens.

8. *Carbohydrate and Lipid Metabolic Effects.* Oral contraceptives have been shown to cause glucose intolerance in a significant percentage of users. Oral contraceptives containing greater than 75 micrograms of estrogens cause hyperinsulinism, while lower doses of estrogen cause less glucose intolerance. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents. However, in the nondiabetic woman, oral contraceptives appear to have no effect on fasting blood glucose. Because of these demonstrated effects, prediabetic and diabetic women should be carefully observed while taking oral contraceptives. A small proportion of women will have persistent hypertriglyceridemia while on the pill. As discussed earlier (see "Warnings" 1a. and 1d.), changes in serum triglycerides and lipoprotein levels have been reported in oral-contraceptive users.

9. *Elevated Blood Pressure.* An increase in blood pressure has been reported in women taking oral contraceptives and this increase is more likely in older oral-contraceptive users and with continued use. Data from the Royal College of General Practitioners and subsequent randomized trials have shown that the incidence of hypertension increases with increasing quantities of progestogens. Women with a history of hypertension or hypertension-related diseases, or renal disease should be encouraged to use another method of contraception. If women with hypertension elect to use oral contraceptives, they should be monitored closely, and if significant elevation of blood pressure occurs, oral contraceptives should be discontinued. For most women, elevated blood pressure will return to normal after stopping oral contraceptives, and there is no difference in the occurrence of hypertension among ever-and never-users.

10. *Headache.* The onset or exacerbation of migraine or development of headache with a new pattern that is recurrent, persistent or severe requires discontinuation of oral contraceptives and evaluation of the cause.

11. *Bleeding Irregularities.* Breakthrough bleeding and spotting are sometimes encountered in patients on oral contraceptives, especially during the first three months of use. The type and dose of progestogen may be important. Nonhormonal causes should be considered and adequate diagnostic measures taken to rule out malignancy or pregnancy in the event of breakthrough bleeding, as in the case of any abnormal vaginal bleeding. If pathology has been excluded, time or a change to another formulation may solve the problem. In the event of amenorrhea, pregnancy should be ruled out. Some women may encounter post-pill amenorrhea or oligomenorrhea, especially when such a condition was preexistent.

Precautions

1. PHYSICAL EXAMINATION AND FOLLOW UP. A complete medical history and physical examination should be taken prior to the initiation or reinstitution of oral contraceptives and at least annually during use of oral contraceptives. These physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including cervical cytology, and relevant laboratory tests. In case of undiagnosed, persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be conducted to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care. 2. LIPID DISORDERS. Women who are being treated for hyperlipidemias should be followed closely if they elect to use oral contraceptives. Some progestogens may elevate LDL levels and may render the control of hyperlipidemias more difficult. (See "Warnings" 1d.) 3. LIVER FUNCTION. If jaundice develops in any woman receiving such drugs, the medication should be discontinued. Steroid hormones may be poorly metabolized in patients with impaired liver function. 4. FLUID RETENTION. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention. 5. EMOTIONAL DISORDERS. Patients becoming significantly depressed while taking oral contraceptives should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug related. Women with a history of depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. 6. CONTACT LENSES. Contact-lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist. 7. DRUG INTERACTIONS. Reduced efficacy and increased incidence of breakthrough bleeding and menstrual irregularities have been associated with concomitant use of rifampin. A similar association, though less marked, has been suggested with barbiturates, phenylbutazone, phenytoin sodium, and possibly with griseofulvin, ampicillin and tetracyclines. 8. INTERACTIONS WITH LABORATORY TESTS. Certain endocrine-and liver-function tests and blood components may be affected by oral contraceptives: a. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. b. Increased thyroid-binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. c. Other binding proteins may be elevated in serum. d. Sex-binding globulins are increased and result in elevated levels of total circulating sex steroids and corticoids; however, free or biologically active levels remain unchanged. e. Triglycerides may be increased. f. Glucose tolerance may be decreased. g. Serum folate levels may be depressed by oral-contraceptive therapy. This may be of clinical significance if a woman becomes pregnant shortly after discontinuing oral contraceptives. 9. CARCINOGENESIS. See "Warnings" section. 10. PREGNANCY. Pregnancy Category X. See "Contraindications" and "Warnings" sections. 11. NURSING MOTHERS. Small amounts of oral-contraceptive steroids have been identified in the milk of nursing mothers and a few adverse effects on the child have been reported, including jaundice and breast enlargement. In addition, oral contraceptives given in the postpartum period may interfere with lactation by decreasing the quantity and quality of breast milk. If possible, the nursing mother should be advised not to use oral contraceptives but to use other forms of contraception until she has completely weaned her child.

Information for the Patient

See Patient Labeling.

Adverse Reactions

An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see "Warnings" section): thrombophlebitis, arterial thromboembolism, pulmonary embolism, myocardial infarction, cerebral hemorrhage, cerebral thrombosis, hypertension, gallbladder disease, hepatic adenomas or benign liver tumors.

There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: mesenteric thrombosis, retinal thrombosis.

The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug related: nausea; vomiting; gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding; spotting; change in menstrual flow; amenorrhea; temporary infertility after discontinuation of treatment; edema; melasma which may persist; breast changes: tenderness, enlargement, secretion; change in weight (increase or decrease); change in cervical erosion and secretion; diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening); intolerance to contact lenses.

The following adverse reactions have been reported in users of oral contraceptives and the association has been neither confirmed nor refuted: congenital anomalies, premenstrual syndrome, cataracts, optic neuritis, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, cerebral-vascular disease with mitral valve prolapse, lupus-like syndromes, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, impaired renal function, hemolytic uremic syndrome, Budd-Chiari syndrome, acne, changes in libido, colitis, sickle-cell disease.

Overdosage

Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Noncontraceptive Health Benefits

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Effects related to inhibition of ovulation: decreased incidence of functional ovarian cysts, decreased incidence of ectopic pregnancies.

Effects from long-term use: decreased incidence of fibroadenomas and fibrocystic disease of the breast, decreased incidence of acute pelvic inflammatory disease, decreased incidence of endometrial cancer, decreased incidence of ovarian cancer.

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Hysteroscopic myometrial biopsy: Its use in diagnosing adenomyosis and its clinical application

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OBJECTIVE: The purpose of this study was to develop and clinically evaluate a hysteroscopic myometrial biopsy for diagnosing adenomyosis.
STUDY DESIGN: Ninety patients with menorrhagia had myometrial biopsies. The depth of adenomyosis was correlated with the severity of menorrhagia.
RESULTS: Of the 90 patients studied, 50 had hysteroscopically normal appearing cavities (i.e., without polyps or submucous myomas). Of those 50, 33 (66%) had significant adenomyosis (>1 mm) as compared with controls (0.8 mm). A statistically significant correlation between the depth of adenomyosis and the severity of menorrhagia was found. ($p = 0.05$).
CONCLUSION: A myometrial biopsy can diagnose adenomyosis. Minimal adenomyosis can be treated definitively by endometrial ablation. Deep adenomyosis should be treated with a hysterectomy. Endometrial glands left under a scar could not only bleed and cause pain but also have malignancy potential. The scar may delay bleeding and the diagnosis of endometrial cancer. Routine myometrial biopsy at the time of operative hysteroscopy should be added to our diagnostic armamentarium. (AM J OBSTET GYNECOL 1992;166:1619-28.)

Key words: Hysteroscopy, myometrial biopsy, adenomyosis, abnormal uterine bleeding, menorrhagia

Adenomyosis has been difficult to diagnose without the surgical pathology of a hysterectomy specimen.

In the past, attempts to diagnose adenomyosis preoperatively with hysterography,[1] magnetic resonance imaging (MRI),[2] or ultrasonography[3] have met with little enthusiasm.

With the advent of hysteroscopy the diagnosis is less elusive. Severe adenomyosis has a typical hysteroscopic appearance of cavities and trabeculations. Milder forms of adenomyosis cannot be diagnosed visually, but require tissue for microscopic examination.

Therefore a myometrial biopsy specimen obtained at the time of operative hysteroscopy was developed and evaluated.

Several questions must be addressed.

Can a single myometrial biopsy specimen be used to adequately diagnose adenomyosis and, if so, is it representative of the entire endometrial cavity?

Is a myometrial biopsy helpful in identifying the cause of menorrhagia in patients with hysteroscopically normal appearing endometrial cavities (i.e., without polyps or submucous myomas)?

Does the depth of endometrial penetration into the myometrium correlate with the degree of menorrhagia and, if so, should the current definition of adenomyosis be changed?

Can future therapy be better tailored if the depth of adenomyosis in patients with persistent menorrhagia is known? Should a hysterectomy be recommended instead of an endometrial ablation in patients with persistent menorrhagia and deep adenomyosis?

Material and methods

Ninety consecutive patients with significant menorrhagia requiring operative hysteroscopy were studied from November 1989 to June 1991.

Most patients had regular ovulatory periods confirmed by either a luteal phase endometrial biopsy showing secretory endometrium or a serum progesterone level in the ovulatory range. All patients found to have dysfunctional uterine bleeding were cycled with progesterone. They were included in the study only if this did not correct the problem. All obese patients and all patients over 40 years of age had endometrial biopsies. Three patients were excluded because their biopsies revealed atypical adenomatous hyperplasia or endometrial cancer.

Each patient received a trial regimen of nonsteroidal antiinflammatory medication before further therapy.

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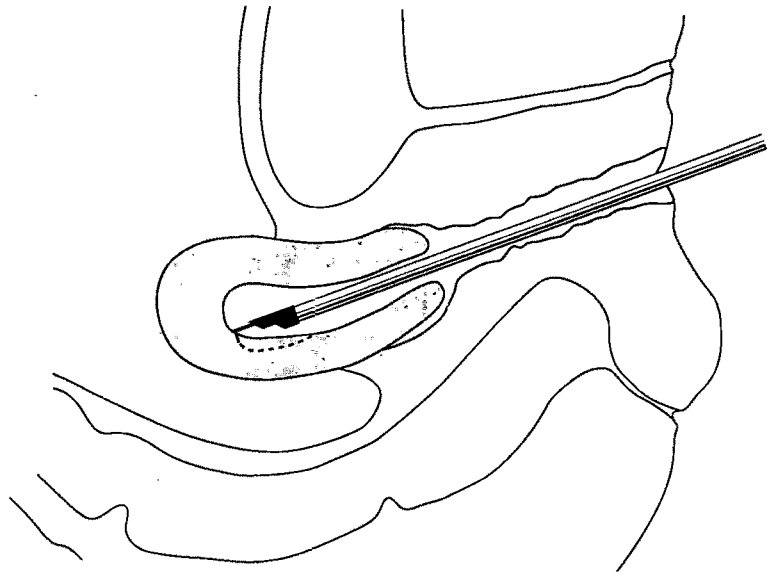


Fig. 1. Myometrial biopsy specimen being taken from the posterior uterine wall during operative hysteroscopy. A 5 mm loop electrode is used to remove a specimen 1.5 to 3 cm long.

A complete blood count and panel of thyroid tests with thyroid-stimulating hormone was included in the evaluation. Two patients were found to have elevated thyroid-stimulating hormone levels. Thyroid replacement corrected their menorrhagia.

Patients with histories of possible bleeding diathesis such as postoperative hemorrhage or easy bruising had complete coagulation work-ups. One patient was diagnosed with von Willebrand disease and had an endometrial ablation.

No office hysteroscopies were performed with this study group.

The amount of bleeding was quantified purely by clot size. Although this may be considered a gross measurement, it reflects enough bleeding to overwhelm the anticoagulant factors in the endometrium. The clot size correlated nicely with the day and night frequency of pad or tampon change in this patient population. Dime-sized clots (1.5 cm) were defined as +; quarter size (2.5 cm), + +; fifty-cent piece size (3 cm), + + +; and silver dollar (4 cm), egg or fist size + + + +. Most patients in the + + + and + + + + categories were unable to carry out their normal daily activities and had anemia.

All operative hysteroscopies were scheduled immediately after a menstrual period and were done with the patient under general anesthesia at the Sutter Surgery Center, an outpatient facility in Sacramento. A laminaria tent was inserted at least 4 hours before the surgery to facilitate cervical dilatation. After the patient was anesthetized the cervix was dilated to 31 French, and a 27 French operative hysteroscope was inserted. The endometrial cavity was thoroughly inspected with sorbitol as the distending medium, and pictures were taken.

If submucous fibroids or endometrial polyps were found they were resected with a loop electrode; a myometrial biopsy was performed followed by a curettage. If only a normal endometrial cavity was visualized, a myometrial biopsy specimen was taken followed by a thorough endometrial curettage. The hysteroscope was reinserted to ensure that all tissue had been removed.

The myometrial biopsy specimen was taken with the 5 mm loop electrode from the posterior wall (Fig. 1). With 70 W of cutting current, the loop was completely embedded 5 mm deep into the posterior myometrial wall near the fundus and a 1.5 to 3 cm segment was resected. The biopsy specimen was tapered up at the end to facilitate visualization of any bleeding sites. Bleeding was controlled with coagulating current through the loop. The biopsied tissue would usually float freely in the endometrial cavity and could easily be grasped with the loop and retracted up against the hysteroscope for removal.

Both posterior and anterior wall myometrial biopsies were performed on 15 patients to correlate the pathologic findings.

Two pathologists, Drs. Anthony Mathios and John Abele, developed a routine for processing the myometrial biopsy specimen and measuring the depth of endometrial penetration.

The specimen, which usually measures 1.5 to 3 cm in length, 5 mm in width, and 5 mm in depth, was completely fixed. The endometrial surface was the flat portion of the specimen, and the myometrial surface was the rounded or convex portion that often showed

variable blackish coagulation markings from the cautery.

The myometrial surface was inked with one of the standard marking dyes, and the specimen was longitudinally sectioned into paired halves by a single cut taken perpendicular to the endometrial surface.

The accuracy of adenomyosis measurements requires proper orientation. The surface to be cut is identified by placing that surface face down on a small orienting sponge within the processing cassette.

With a microscope fitted with a micrometer, the distance between the base of the deepest penetrating gland and the endometrial-myometrial junction is measured. This distance is recorded and mentioned in the final diagnosis.

Thirty consecutive hysterectomy specimens obtained at Sutter Memorial Hospital during the same time period were studied and used as controls. They were matched with the study group by age, gravida, and parity. These patients had histories of normal periods and were having surgery for prolapse, pain, endometriosis, fibroids, or cervical or ovarian tumors. The clot size, if any, could not be determined because this was a chart review. For consistency Dr. Mathios did all measurements for the study group and controls.

Nonparametric statistical analysis was performed.

Results

Ninety consecutive patients with menorrhagia had myometrial biopsies performed at the time of operative hysteroscopy. Of these, 50 had normal-appearing cavities, 27 had gross endometrial polyps, and 13 had submucous myomas.

Table I lists the 50 patients with normal-appearing endometrial cavities. The group is organized by depth of endometrial penetration into the myometrium, with the deepest listed first. Each patient's age, gravida, parity, and amount of menorrhagia are included. There is a good correlation between the amount of menorrhagia (clot size) and the depth of endometrial penetration. This penetration ranged from 6 to 0 mm. The mean depth in patients with + bleeding was 0.84, + + 1.44 mm, + + + 2.98 mm, and + + + + 2.35 mm.

Nonparametric statistical tests showed that a woman with a grossly normal endometrial cavity who passed clots the size of a quarter or larger is 2.9 times as likely to have meaningful adenomyosis (more than 1 mm endometrial penetration) than a woman who has not passed such large clots. This difference is highly significant. The clot size does not correlate with the depth of adenomyosis in women with polyps or myomas seen at hysteroscopy.

Table II lists the control group with normal periods whose members were matched by age, gravida, and parity with the study group. This control group is organized by depth of endometrial penetration. The depth of endometrial penetration in the anterior and posterior wall has been separately listed along with the uterine weight. The depth of penetration ranged from 1.5 mm to 0 mm. The average depth of penetration in the posterior wall (mean 0.8 mm) was almost always greater than that in the anterior wall (mean 0.46 mm). The group of women with heavy menstrual periods had significantly greater posterior wall depth than women with normal periods. The average posterior wall penetration in the women with menorrhagia was nearly twice the average depth among women with normal menstrual periods. There appeared to be a shift toward extremely heavy periods (+ + + and + + + + +) when 1 mm penetration had occurred in the study group. Because the mean endometrial penetration of the posterior wall in the control group was 0.8 mm, 1 mm was selected as the depth that could potentially cause menorrhagia and was defined as significant adenomyosis.

Table III lists the 27 patients who had gross endometrial polyps. After the polyps were resected a myometrial biopsy was performed to measure the amount of endometrial penetration in these patients. Fourteen (52%) had significant penetration; however, this could not be correlated with the amount of bleeding, suggesting that the polyps are the main cause of the menorrhagia.

Table IV lists the 13 patients who had submucous myomas. After the myomas were resected, a myometrial biopsy was performed to measure the amount of endometrial penetration. Eight (61%) had significant penetration but, like the polyp group, this could not be correlated with the amount of bleeding, suggesting that the submucous myomas are the main cause of the menorrhagia. The heaviest bleeding with the largest clots and frequent anemia was in the fibroid groups.

Comment

Can a myometrial biopsy diagnose adenomyosis? This study confirms that the diagnosis of adenomyosis with endometrial penetration ≤6 mm in depth can be made with a myometrial biopsy.

Is a single posterior myometrial biopsy representative of the entire endometrial cavity? This question must be answered for this study to have validity. When diffuse adenomyosis is present, the posterior uterine wall is the area most frequently and severely affected, a fact that has been known for years and was confirmed recently with newer imaging techniques.

In 1896 von Recklinghausen[4] observed that the glandular elements in adenomyosis were more commonly found in the posterior wall of the uterus. This has been confirmed by Benson and Sneeden,[5] who found adenomyosis to be a diffuse process most frequently in the posterior wall, less frequently in the anterior wall, and rarely involving only the cornu or portions of the uterus

Table I. Hysteroscopically normal cavity

Patient	*Age*	*Gravida*	*Para*	*Depth* (mm)*	*Menorrhagia*
MM	46	2	2	6	+ + +
MN	44	2	2	5.4	+ + + +
VB	47	3	3	5	+ + +
JD	46	1	1	3.7	+ + + +
BC	53	5	4	3.6	+ + + +
AP	44	2	1	3.5	+ +
KW	39	3	2	3	+ + +
LC	42	3	2	3	+ + +
PW	45	3	3	2.8	+ + + +
KH	37	1	1	2.6	+ + + +
LP	43	5	0	2.2	+ + +
SE	43	3	2	2.1	+ + + +
JU	46	4	3	2.1	+ + + +
LB	46	5	4	2	+ + + +
YD	41	3	2	2	+ +
YW	45	2	2	2	+ + +
CD	41	5	3	2	+ +
DR	37	3	1	2	+ + + +
JP	28	3	2	1.8	+ + + +
JP	30	5	2	1.8	+ − +
NC	48	2	2	1.5	+ − +
SR	34	3	3	1.5	+ − +
JW	31	3	3	1.4	+ + + +
JF	43	0	0	1.4	+ +
KA	28	2	2	1.2	+
LM	28	4	4	1.2	+ +
BU	38	2	1	1.2	+ + + +
SL	38	1	0	1	+ +
EA	45	2	2	1	+ + + +
RR	38	2	2	1	+
KK	39	3	0	1	+
LM	35	1	1	1	+
AH	24	2	2	1	+
SC	31	3	3	0.9	+
BZ	37	4	3	0.8	+
LL	41	4	4	0.8	+
BD	42	3	2	0.8	+ +
VA	39	2	1	0.7	+
KB	32	1	0	0.7	+
RB	36	2	2	0.7	+ +
SS	43	2	2	0.6	+
MJ	43	6	4	0.6	+
MR	37	6	4	0.6	+
JT	43	5	1	0.5	+
MT	44	1	1	0.4	+ +
SS	30	3	3	0.3	+ +
MJ	31	2	1	0.3	+
RS	48	2	2	0	+ +
WS	34	2	2	0	+
KJ	40	0	0	0	+

*Depth (mm) of endometrial penetration into the myometrium.

near the internal os. Emge[6] found endometrial invading the posterior wall more commonly than the anterior or lateral wall. Novak and Woodruff[7] reported that adenomyosis is mainly a diffuse process; as a rule the posterior wall is more severely involved. Azziz[8] states that the posterior wall of the uterus is usually involved to a greater degree by the adenomyotic process and is consequently thicker in these uteri.

The newer imaging techniques have confirmed the above findings. Bohlman et al.[3] with ultrasonography and Togashi[2] with MRI each studied adenomyosis and found it to be diffuse, with the posterior wall most severely affected.

In my study hysteroscopic anterior and posterior myometrial biopsies were performed on 15 patients. Thirty patients in the control group also had the depth of endometrial penetration measured in both the anterior and posterior uterine walls, the areas routinely sampled (Table II). In both groups adenomyosis was found to be diffuse and to involve the entire uterine

Table II. Controls: Normal periods

Patient	Age (yr)	Gravida	Para	Depth* (mm)		Uterine weight (gm)	Diagnosis
				Anterior	Posterior		
EC	40	3	3	1.1	1.5	144	CIS
SB	30	3	1	.8	1.5	60	Prolapse
KL	33	4	2	.9	1.4	65	CIS
PB	35	4	3	1	1.4	145	Pain
GS	50	2	2	.7	1.3	194	Cyst
JW	29	6	3	.6	1.3	127	Prolapse
JS	34	3	2	.8	1.3	105	CIS
JK	38	2	2	1.1	1.3	111	Prolapse
TP	36	2	1	—	1.3	125	Pain
LF	43	4	3	1	1.2	150	CxCa
LL	31	3	2	—	1.2	115	Pain
EP	45	0	0	.5	1.1	163	CxCa
NT	40	2	2	1	1.1	121	Pain
DT	36	3	3	.9	1.1	136	CxCa
GB	44	2	2	0	.9	175	Prolapse
LS	33	0	0	—	.9	88	CIS
PR	29	5	3	.7	.9	132	CIS
CB	27	1	1	0	.9	142	Pain
AB	41	0	0	.5	.7	71	Cyst
CL	29	4	3	.7	.6	127	Pain
MB	36	4	4	.4	.6	97	Prolapse
LK	45	4	3	0	.5	235	Fibroid
LS	37	2	2	.3	.4	91	Prolapse
EJ	46	2	0	.4	.3	630	Fibroid
MP	48	3	3	0	0	170	Prolapse
MV	43	2	1	.6	—	72	Prolapse
SG	42	3	3	0	0	165	CxCa
KC	31	0	0	0	0	70	CxCa
LL	29	4	3	0	0	99	CIS
CK	25	3	3	0	0	111	Pain
MEAN DEPTH				0.46	0.8		

Cyst, Ovarian cyst; *CxCa,* cervical cancer; *Pain,* pelvic pain; *CIS,* cervical carcinome-in-situ; *Prolapse,* uterine or vaginal wall prolapse; *Fibroid,* uterine leiomyoma.

*Depth of endometrial penetration into the myometrium in the anterior and posterior uterine walls. Anterior wall mean = 0.46 mm; posterior wall mean = 0.8 mm.

cavity. Both the anterior and posterior walls were affected, but the deepest penetration was almost always in the posterior wall.

Therefore a *single* myometrial biopsy of the posterior uterine wall is not only diagnostic of adenomyosis but also represents the area most severely involved (with the deepest endometrial penetration into the myometrium).

Can a myometrial biopsy be helpful in evaluating menorrhagia when a normal cavity is visualized hysteroscopically? It is perplexing to find a hysteroscopically normal-appearing intrauterine cavity in patients with significant menorrhagia. Unfortunately, this is a common scenario.

Of the 90 patients evaluated for menorrhagia, 50 had essentially normal-appearing cavities without gross polyps or submucous myomas (Table I). Although many had abundant tissue in spite of being postmenstrual, the term "hyperplasia" was not used because it is hysteroscopically difficult to make this diagnosis. Instead, in this study these cases were placed in the normal-cavity category. Twenty-seven cases were found to have gross polyps (Table III), and 13 had submucous myomas (Table IV).

Other investigations in which hysteroscopy was used to study menorrhagia have described a similar experience (Table V).

Fraser[9] found that 94 (51%) of the 182 patients with menorrhagia had grossly normal-appearing cavities hysteroscopically. Sixteen (9%) had submucous myomas, and 29 (16%) had polyps.

Loffer[10] studied 187 patients with abnormal uterine bleeding. Of the 102 having pure menorrhagia, 68 (66%) had normal cavities. Thirteen (13%) had polyps, and 16 (16%) had fibroids.

In evaluating menorrhagia in a patient with a normal-appearing endometrial cavity, a myometrial biopsy appears to be helpful.

Table III. Hysteroscopic endometrial polyps

Patient	*Age*	*Gravida*	*Para*	*Depth* (mm)*	*Menorrhagia*
AA	45	3	3	3	++++
CT	46	3	3	2.9	+++
AF	53	3	3	2.7	+++
CH	45	2	2	2.1	++
BW	40	1	1	2	++
KZ	44	4	4	1.8	+++
PG	46	2	2	1.7	+
NS	48	3	1	1.7	+++
SK	46	2	2	1.5	++++
FC	47	1	1	1.5	+++
DC	48	3	3	1.1	++
JV	40	8	3	1	++++
LT	38	2	2	1	+++
LL	37	4	3	1	++
NH	35	2	2	.9	++
JR	45	3	3	.5	−
CL	36	4	2	.5	++++
LK	47	3	2	.4	++
MM	38	2	0	.4	++++
SW	27	0	0	.4	++
MW	40	0	0	.4	++
ZF	47	3	2	.3	++++
KG	47	2	2	.3	++
KS	35	2	1	.2	++++
RW	48	2	2	0	++
NK	31	1	1	0	++
ND	43	1	1	0	++

*Depth of endometrial penetration into the myometrium.

Table IV. Hysteroscopic submucous myoma

Patient	*Age*	*Gravida*	*Para*	*Depth* (mm)*	*Menorrhagia*
PT	40	2	2	2.5	++
ML	46	2	2	1.9	+++
CO	45	1	1	1.8	+
MS	44	0	0	1.5	++++
SH	51	3	3	1.5	++
DS	33	2	2	1.3	++++
GC	50	1	1	1.1	++
LW	42	3	2	1	+
EC	43	2	2	.8	+++
KS	34	2	1	.6	++++
JW	45	3	2	.3	++++
JM	42	3	1	0	+
JW	45	3	2	0	++++

*Depth of endometrial penetration into the myometrium.

In my study 33 of 50 (66%) patients with normal cavities had significant adenomyosis (endometrial penetration >1 mm) as compared with controls (0.8 mm), which accounts for their menorrhagia. If heavy bleeding persists after a thorough curettage, the diagnosis of adenomyosis can explain the problem.

Of the 27 patients with gross polyps and the 13 with submucous myomas, 14 (51%) and 8 (29%) also had significant adenomyosis. If these patients continue to bleed abnormally after their polyps or myomas are successfully resected, adenomyosis may explain the problem.

Does menorrhagia correlate with the depth of endometrial penetration into the myometrium? In this study there was a statistically significant correlation between the amount of menorrhagia (clot size) and the depth of endometrial penetration when the uterine cavity appeared normal hysteroscopically.

A review of the world literature reveals only one publication addressing the possibilities that minimal endometrial penetration can cause abnormal bleeding.

Bird et al.[11] (1972) coined the term "adenomyosis subbasalis" and defined it as adenomyosis within one low power field (2 mm) below the "basal" endometrium but with no further penetration. Of the 47 patients with this and no other pathologic condition (i.e., polyps or fibroids), 28 (60%) had significant menorrhagia. Bird et al. concluded that adenomyosis subbasalis is an im-

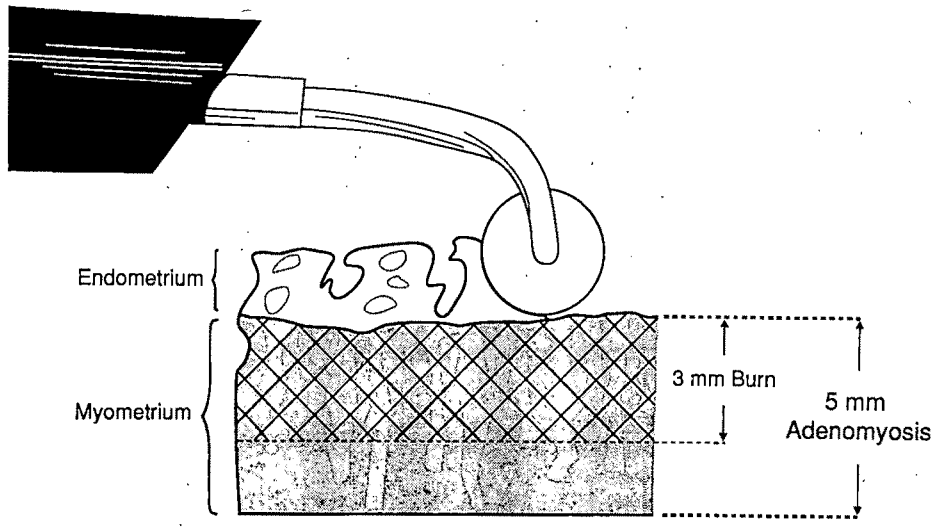


Fig. 2. Three-millimeter burn by roller-ball ablation potentially leaving endometrial glands under the scar in a patient with 5 mm deep adenomyosis.

portant symptom-producing entity and that the depth of penetration correlated with the occurrence of menorrhagia.

In an unpublished report (personal communication) Richard H. Oi, a board-certified anatomic pathologist and obstetrician-gynecologist, studied the endometrial myometrial junction in 67 patients who had had hysterectomies for prolapse, stress incontinence, and pelvic relaxation with no other uterine pathologic condition. He found an "exaggerated down growth" of endometrium into the myometrium in 31 cases, and all but four of these women had menorrhagia. Of the 36 cases with no down growth, 27 had normal periods without menorrhagia. Pathologists usually sign out these uteri as "normal." Oi demonstrated that an "exaggerated endometrial down growth" can be symptomatic.

Prognostic and future therapeutic value when adenomyosis is diagnosed by myometrial biopsy. If menorrhagia persists or recurs after a thorough curettage in a patient with a hysteroscopically normal appearing cavity or after complete resection of polyps or submucous myomas, a myometrial biopsy showing adenomyosis at the time of surgery can explain the problem.

A myometrial biopsy can demonstrate the depth of endometrial penetration, which may have prognostic value and allow the physician to tailor future therapy.

Persistent menorrhagia caused by adenomyosis in women who desire no more children can be definitively treated by either an endometrial ablation or a hysterectomy. Minimal endometrial penetration can be treated successfully with an ablation. However, it may be more prudent to treat deep adenomyosis with a hysterectomy. Endometrial glands left under a scar may cause recurrent bleeding and pain (Fig. 2). Townsend (personal communication) has performed approximately 10 hysterectomies for bleeding or pain after an endometrial ablation, and almost all have had deep adenomyosis.

Table V. Hysteroscopic findings in patients with menorrhagia

	Fraser		*Loffer*		*McCausland*	
Findings	*No.*	*%*	*No.*	*%*	*No.*	*%*
Total	182	—	102	—	90	—
Normal cavity	94	51	68	66	50	55
Polyps	29	16	13	13	27	30
Submucous myoma	16	9	16	16	13	15

The other long-term concern of leaving an endometrial gland under a scar is that if the ectopic gland becomes malignant, the scar could delay bleeding and the diagnosis of endometrial cancer.

This is not just a theoretical concern. Novak and Woodruff[7] state that atypical hyperplasia is not uncommonly seen in deep islands of ectopic endometrium. They reviewed the world literature of cases in which endometrial adenocarcinoma originated in the aberrant glands. They feel that this problem may be more common than reported because malignant adenomyosis may creep up to the endometrial surface and the pathologist may understandably diagnose it as endometrial carcinoma with myometrial extension.

Woodruff et al.[13] reported two cases of adenocarcinoma arising in adenomyosis diagnosed by atypical vaginal cytology. Unfortunately, postablation scarring could also prevent this diagnostic tool.

Complications of myometrial biopsies. There were no immediate or long-term complications of myometrial biopsies. Bleeding from the biopsy site can be bothersome, but coagulating the bleeder with the loop or rollerball electrode is usually successful. In one case it was necessary to inject vasopressin paracervically. No perforations occurred and are unlikely if proper technique is followed. A premenopausal uterine wall is between 1.5 and 2 cm (15 to 20 mm) thick. The loop electrode used for the biopsy is only 5 mm deep. Although hysteroscopic magnification makes it appear deeper, only one quarter to one third of the wall is being biopsied.

A myometrial biopsy should not be performed on a postmenopausal uterus, which may have a thin wall. It adds little in the evaluation of postmenopausal bleeding because adenomyosis is usually not a factor.

Ten hysterectomies have been performed for persistent menorrhagia 3 to 9 months after myometrial biopsy. There were no uterine wall defects seen at the biopsy site and no intrauterine adhesion.

In conclusion, a myometrial biopsy specimen can be used to diagnose adenomyosis. If taken from the posterior wall, it represents not only the entire endometrial cavity but also the area most severely involved.

The depth of endometrial penetration correlated closely with the degree of menorrhagia when the uterine cavity appeared normal hysteroscopically (i.e., without gross polyps or submucous fibroids).

A myometrial biopsy to determine endometrial adenomyosis has clinical significance. First, it helps the clinician make a more accurate diagnosis when evaluating menorrhagia, especially in patients with hysteroscopically normal-appearing intrauterine cavities. If a thorough curettage fails to correct the menorrhagia, a myometrial biopsy showing adenomyosis at the time of the surgery clarifies the issue.

Second, if menorrhagia persists after complete hysteroscopic resection of endometrial polyps or submucous fibroids, a myometrial biopsy showing adenomyosis can also explain this dilemma.

Third, a myometrial biopsy can demonstrate the depth of penetration that correlates closely with the amount of menorrhagia and allows the physician to tailor future therapy. If menorrhagia persists, minimal penetration may be treated definitively by an endometrial ablation. However, it may be more prudent to treat deep adenomyosis with a hysterectomy. Endometrial glands left under a scar could not only bleed and cause pain but also have future malignancy potential. The scar may delay bleeding and the subsequent diagnosis of endometrial cancer.

Routine myometrial biopsy at the time of operative hysteroscopy should be added to our diagnostic armamentarium.

I thank Drs. John Abele, Dan Egerter, Gary Fields, Charlie Gamble, France Holmes, Anthony Mathios, Richard Oi, Steven Samuel, and Duane Townsend for their valuable assistance. I also thank Vance McCausland for his technical help in preparing this paper.

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Editor's note: This manuscript was revised after these discussions were presented.

Discussion

DR. WILLIAM K. GRAVES, San Francisco, California. I am pleased to discuss this paper because of my longstanding interest in this entity. I was the formal discussant for Dr. John Molitor's presentation of the subject before this society in 1970.[1]

Today Dr. McCausland presented 90 patients with significant menorrhagia in whom dilatation and curettage was indicated. Anovulatory bleeding was excluded or had failed to respond to cyclic progesterone therapy. Thyroid and hematologic disorders were ruled out by appropriate tests. Adenomatous hyperplasia and endometrial cancer had been excluded by endometrial biopsy in all patients >40 years old or who were obese. Most subjects had received a trial regimen of prostaglandin synthetase inhibitors. At the time of the dilatation and curettage, hysteroscopy was performed; this is a combination that we increasingly recognize as ap-

propriate. During the hysteroscopy Dr. McCausland took a myometrial biopsy specimen of the posterior uterine wall with a 5 mm loop electrode. This area has been recognized since the initial descriptions of adenomyosis as being most frequently involved by adenomyosis.

The great value of this study is that it includes only cases with menorrhagia. Whether the incidence of adenomyosis in women in the 40- to 50-year age range is as low as 6% or as high as 60%,[2] it is clear that it is not the presence of endometrial glands in the myometrium per se that is clinically meaningful, it is the associated symptoms. In his retrospective study of hysterectomy cases,[1] Dr. Molitor judged that in 40% of the cases adenomyosis was the primary cause of the patient's symptoms and that it was a contributing cause in another 30%. In the remaining 30%, adenomyosis was an incidental finding.

The first of my two questions is, what is the effectiveness of myometrial sampling as a diagnostic method compared with sonography, hysteroscopy alone, and MRI?

Irregular translucent myometrial zones seen on ultrasonography had been reported as being characteristic of adenomyosis. However, other reports attest to the difficulty of sonographic diagnosis of adenomyosis. For example, a retrospective report of 80 patients who had sonography before hysterectomy showed the sensitivity of sonography in distinguishing adenomyosis from leiomyomas to be 63% and the specificity to be 97%.[3] It seems to me unlikely that the vaginal probe will add much precision to this determination.

Because endometrium overlying an area of adenomyosis is often normal, making the diagnosis by hysteroscopy alone would also seem to be a hit-or-miss proposition. Dr. McCausland's data support this conclusion because 50 of his menorrhagia patients had grossly normal cavities at hysteroscopy, but two thirds of them had endometrial penetration >1 mm.

Reports of the effectiveness of MRI in differentiating adenomyosis from leiomyomas are encouraging. In a retrospective study of 93 patients with enlarged uteri,[4] MRI showed 71 had myomas, 16 had adenomyosis, and 6 had both. The MRI diagnosis was confirmed by surgery and pathologic examination in 92 of the 93 cases. In a prospective study[5] of 21 premenopausal patients with clinical diagnoses of adenomyosis, eight cases were actually adenomyosis as demonstrated by histologic findings from the hysterectomy specimen; all eight were correctly diagnosed by MRI. Another 12 had leiomyomas, 5 of which had microscopic foci of adenomyosis; 10 of the 12 were correctly diagnosed. In two cases microscopic foci of adenomyosis in myomas were not demonstrated by MRI. Although expensive, this noninvasive diagnostic method is accurate.

My second question is, to what extent is confirmation or exclusion of the diagnosis of adenomyosis necessary or even useful in management of the women with significant menorrhagia? A skeptic might say that the important considerations are the severity of bleeding and pain and the extent to which they interfere with overall health and quality of life. Only of secondary importance is whether the cause is myomata or adenomyosis. A skeptical attitude may have been reasonable 5 to 10 years ago when hysterectomy was the only treatment under consideration. However, in this age of gonadotropin-releasing hormone agonists, myomectomy, and endometrial ablation procedures, a precise diagnosis is mandatory.

Dr. McCausland expressed concern about endometrial ablation in patients with deep endometrial penetration. In an era of widespread use of hormone replacement therapy after menopause, a depth of adenomyosis >2.5 mm may be a contraindication to endometrial destruction procedures. Conversely, the absence of adenomyosis in a posterior wall biopsy supports the use of ablation if the patient so desires. It may be that myometrial biopsy should be a prerequisite for endometrial ablation when menorrhagia is the primary indication.

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Dr. Joseph A. Oliver, Pasadena, California. Do you do office dilatation and curettage and hysteroscopy? Why or why not?

Dr. Lyman Rust, Hanford, California. I am glad you mentioned the problem of deep adenomyosis and the effect endometrial ablation might have in covering up endometrial tissue. It has bothered me for some time to know that there are a lot of gynecologists who rollerball patients all over the place before we know what the long-term effects are. Dr. McCausland, did you suppress these patients before hysteroscopy with danazol or a gonadotropin-releasing hormone analog? Might that affect on your biopsy results?

Dr. James A. Merrill, Seattle, Washington. It has puzzled me why adenomyosis causes menorrhagia, if indeed it does. Dr. McCausland, do you have an answer? In my experience in the pathology laboratory adenomyosis is equally often an incidental finding not occurring in a patient with symptoms of menorrhagia. Treating patients for this disorder on the basis of a biopsy diagnosis represents a considerable step forward.

Dr. Hampton W. Irwin, Spokane, Washington. Dr. Philip Brooks presented a paper last year in which he described suppression by gonadotropin-releasing hor-

mone agonist and other methods to prepare for endometrial ablation.[1] Using the same electroresection instrument that Dr. McCausland used, Dr. Brooks takes strips 4 to 5 mm in depth from most of the fundal surface and uses the rollerball for areas not adequately treated with the resectoscope. I have used that technique, and our pathologist frequently reports adenomyosis in specimens from ablations.

Dr. McCausland, your report inspires me to review my cases to find the correlation of histologic findings, history, and outcome. I expect that deeper ablation with electroresection will prove to be more effective in patients with adenomyosis than use of the rollerball alone.

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DR. MCCAUSLAND (Closing). Dr. Graves, I am sure that there are cases of asymptomatic adenomyosis, but I feel that the prevalence is overstated. Almost all past studies have been retrospective chart reviews of patients found to have adenomyosis at hysterectomy. If the chart said that the menstrual periods were normal, the authors classified the case as asymptomatic. When reviewing the charts of those in the hysterectomy control group for this paper, I found how inaccurate a menstrual history from a hospital chart is likely to be. Some of these patients with "normal" periods were anemic with hemoglobin counts of 10. I called the physicians who did the hysterectomy and asked whether the periods were really normal. For example, after consulting the office record they said, "Well, they were somewhat heavy, but I was doing the surgery for stress incontinence so I just put down that the periods were okay." I question whether chart reviews for menstrual history are valid unless the author personally talked to the patient. Therefore, the incidence of "asymptomatic" adenomyosis is likely to be overstated.

Dr. Oliver, I do not think that office hysteroscopy and dilatation and curettage help much in patients with significant menorrhagia. You first need to make sure that the patient has adequate progesterone exposure by either doing an endometrial biopsy or a serum progesterone test. If in the midluteal phase the progesterone is <10 ng/ml or the endometrial biopsy specimen shows proliferative endometrium, I give the patients a 3-month course of cyclic progesterone. If this does not correct the problem I schedule an operative hysteroscopy. An office procedure at that time would be of little value for polyps or submucous myomas that need to be resected. I now feel that a patient with a grossly normal cavity needs a myometrial biopsy and a vigorous curettage. I am not talking about a wimpy suction dilatation and curettage in the office. I make sure that the endometrial lining is all out by looking afterward. If it is not, I curette again until it is. The patients who respond to this type of dilatation and curettage are those who have a normal cavity without adenomyosis. I think they have an abnormally functioning endometrium. Remember that I do these procedures immediately after the mentrual period. This is the best time to do operative hysteroscopy because at later phases there is so much build-up of endometrial tissue that finer details are obscured. Just after the menstrual period, at hysteroscopy, it is expected to see a thin lining that shows early proliferative histology. However, in most of these patients I found abundant tissue and the histology showed mixed estrogenic and progestational response, dyssynchronous endometrium, or unopposed estrogen effect. My working theory is that part of the bleeding problem in many of these patients is an abnormally functioning endometrium, perhaps some type of progesterone receptor defect.

I have not had much time for follow-up since I started this study in November 1989. However, I called my patients just before the meeting. Those with a normal cavity and <1 mm penetration by endometrium have all had normal periods since the dilatation and curettage. The ones with deeper penetration have continued to have problems, and those with the deepest penetration have had either hysterectomy or endometrial ablation. Early in this experience, I did ablations on women with deeply penetrating adenomyosis, but I found that the ones with deep adenomyosis and ablation continued to have pain or bleeding, and most have finally needed a hysterectomy.

Dr. Rust, none of these patients had hormonal suppression before the operative hysteroscopy.

Dr. Merrill, I think there are two reasons patients with adenomyosis bleed. I have already discussed the first, abnormally functioning endometrium. The other may be some type of injury to the endometrium by childbirth, endometritis, or curettage that activates the endometrium to begin penetrating into the myometrium. Of course, there is more surface area to bleed, but even more important is the fact that the endometrial penetration replaces some of the myometrial fibers. Thus the uterus does not contract effectively enough to stop bleeding.

Dr. Irwin, endometrial ablation by resection may be a good procedure for patients who have mild adenomyosis. However, technically it is hard to resect certain parts of the endometrial cavity, especially around the cornu, and you may leave some glands in that area. Using the resectoscope is definitely more dangerous than using a rollerball. There have been four deaths from uterine perforation with endometrial resections (personal communication, 1991, Duane E. Townsend, MD, Davis, California). A rollerball is easier and safer, and it should be effective, in obliterating penetration at the 1 to 2 mm range.

Neonatal morbidity according to gestational age and birth weight from five tertiary care centers in the United States, 1983 through 1986

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San Francisco, California, Birmingham, Alabama, Columbus, Ohio, and Houston, Texas

OBJECTIVES: This study details the incidence, by gestational age and birth weight, of specific neonatal morbidities in singleton neonates without major congenital anomalies.

STUDY DESIGN: Data were prospectively collected on all deliveries at five tertiary centers in the United States during the years 1983 through 1986. Pregnancies were meticulously dated and the gestational ages of the neonates at delivery were confirmed by Dubowitz score.

RESULTS: The incidence of respiratory distress syndrome gradually decreases with increasing gestational age until 36 weeks. A marked decrease in the incidence of necrotizing enterocolitis, patent ductus arteriosus, intraventricular hemorrhage, and sepsis occurs after 32 completed weeks. The number of days of mechanical ventilation for respiratory distress syndrome and newborn stay in the tertiary care facility also were significantly reduced after 32 weeks.

CONCLUSIONS: The incidence of both respiratory distress syndrome and patent ductus arteriosus is markedly decreased by both increasing gestational age and birth weight. The incidence of grade III and IV intraventricular hemorrhage, necrotizing enterocolitis, and sepsis virtually vanishes after 34 weeks. These data relating neonatal morbidities to gestational age are important to the obstetrician in the critical decision regarding the timing of delivery and to the parents, who can benefit from a realistic prediction of the neonatal course. (AM J OBSTET GYNECOL 1992;166:1629-45.)

Key words: Neonatal morbidity, gestational age, respiratory distress syndrome, intraventricular hemorrhage, sepsis

Decisions about the timing of preterm birth are made daily in our tertiary obstetric units. It is essential that the obstetrician making these decisions has information about the incidence of specific neonatal morbidities with respect to gestational age. Balancing the consequences of a preterm delivery with its attendant neonatal morbidities against the maternal risks of continuing a complicated pregnancy is a very complex process. For instance, in the case of a mother with preterm labor the issues include whether to treat the labor, the length of treatment, the choice of single or multiple tocolytic agents, the risk of potentially life-threatening side effects of tocolytic medication on the mother,[1] and the possibility of masking a serious maternal condition (e.g., chorioamnionitis) by treating the preterm labor. The decision process is based on the principle of delivering the neonate with the least morbidity without placing the mother at excessive risk. Clearly, a key element in this decision process is an accurate prediction of a gestational age–specific neonatal morbidity.

In previous reports the incidences of neonatal morbidities are most commonly categorized according to birth weight interval.[2] If the incidence is presented by gestational age, there is usually no notation about the accuracy of prenatal dating. However, the obstetrician making the decision about the timing of a preterm birth usually has more confidence in the gestational age than in the estimated fetal weight. Even when fetal weight is estimated by ultrasonography, there can be complicating factors affecting the accuracy of the estimate, such as presence of ruptured membranes, the inherent error of the various algorithms for estimating fetal weight, inability to attain certain measurements secondary to fetal position, and the lack of sophistication

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Table I. Summary of exclusions

Reason for exclusion	*No. of neonates*
Maternal exclusions	3004
Multiple gestation	695
Neonatal exclusion	499
Stillbirth	301
Both maternal and neonatal exclusion	148
Neonatal death <24 hr	85
Outcome unknown	4
Maternal complications not coded	19
Newborn complications not coded	2
Maternal exclusion data incomplete	21
Neonatal exclusion data incomplete	4
Gestational age <23 wk	9
Gestational age >45 wk	1
Birth weight <500 gm	3
Birth weight unknown	6
TOTAL	4801

Table II. Specific maternal exclusions

Maternal complication	%
Preeclampsia-eclampsia	38.3*
Chronic hypertension	23.4
Diabetes (class A)	18.4
Diabetes (classes B to R)	6.5
Substance use	9.2
Syphilis	2.2
Placenta previa	2.2
Abruptio placentae	6.5
Hemoglobinopathy	1.4
Other (e.g., renal disease, fetal surgery)	3.7

*Exceeds 100% because some patients had more than one exclusion.

of available equipment. This paper emphasizes the incidence of selected neonatal morbidities at specific gestational ages in 20,680 carefully dated, uncomplicated pregnancies. Some of the data will also be described by birth weight, an approach that may be more useful to pediatricians, neonatologists, and hospital administrators. Selected major morbidities will also be described in categories combining birth weight and gestational age.

Methods

Maternal demographic and neonatal outcome data from five tertiary care centers in the United States were obtained from 1983 through 1986. Patients included in this analysis had a last normal menstrual period between November 1, 1982, and December 9, 1985. The data were collected in conjunction with a clinical trial of a preterm birth prevention program in high-risk mothers sponsored by the March of Dimes Birth Defects Foundation. The five centers were the University of Alabama in Birmingham (University Hospital and Cooper Green Hospital), University of California Medical Center at San Diego, Vanderbilt University (Nashville General Hospital, Vanderbilt University Hospital), Ohio State University, and Northwestern University Medical Center. The University of California Medical Center at San Francisco served as the site of data management and analysis. Virtually all pregnant women (low and high risk for preterm labor) who used these centers for their prenatal care were included. However, each center was allowed to determine certain categories of patients to be excluded from the study. Examples include private patients, patients being followed in special clinics for medical complications of pregnancies, such as diabetes, and patients entered into other protocol studies. Maternal and neonatal transports to the centers were excluded at all centers.

Patients were managed according to policies and procedures extant at each center. In general, patients with ruptured membranes did not receive tocolytic drugs. Regardless of the status of the membranes, most patients did not receive corticosteroids.

A delivery data form was completed for each patient at the time of delivery. This form was sent to the University of California Medical Center at San Francisco for data entry 30 days after delivery regardless of whether the neonate was still hospitalized. Data was entered and edited by means of a commercially available information system and a mainframe computer. Internal logistic quality control mechanisms were built into the data entry mechanisms. Any cases not passing the quality control screens resulted in the forms being sent back to the individual sites for review and resubmission. A nurse-clinician from the medical center visited each study site annually to review adherence to protocol and data abstraction. At each visit a random sample of records were reabstracted and compared with the original data.

Determination of gestational age was made meticulously. Assigned prenatal gestational age was based on date of the last menstrual period, the clinical exam at the first visit, and ultrasonography, if performed. A neonatal gestational age was assigned with a modified Dubowitz examination. Each principal investigator reviewed the gestational age assignments of neonates weighing <2750 gm. If there was <3 weeks discrepancy between the prenatal gestational age and the assigned neonatal gestational age, the prenatal gestational age was the final gestational age. If there was a discrepancy of ≥3 weeks, or ≤2 weeks spanning the thirty-seventh week, a preassigned independent obstetrician-observer not otherwise associated with the study assigned the final gestational age after review of all appropriate dating parameters.

From the population of 25,481 we created a subpopulation with the following criteria: (1) live-born, (2) singleton pregnancy, (3) inborn at tertiary care fa-

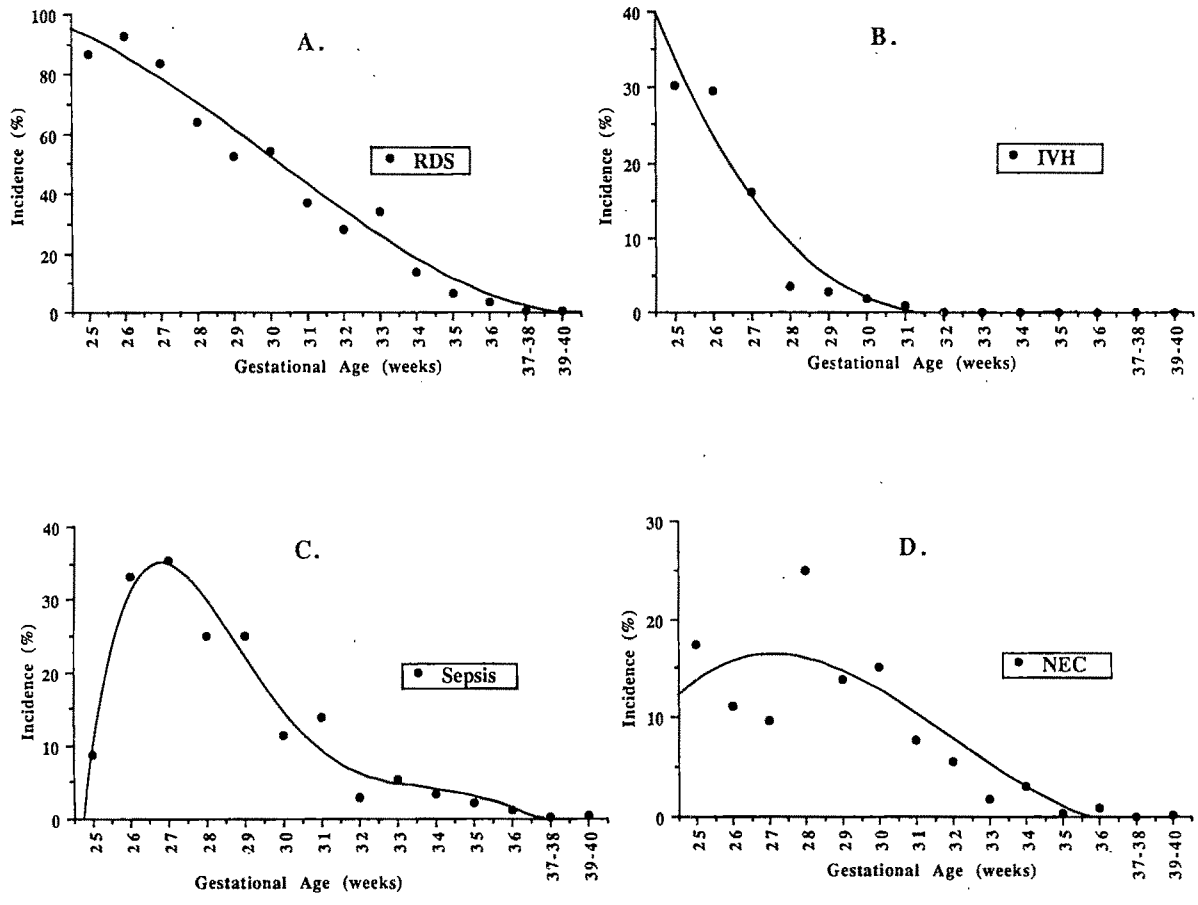


Fig. 1. Incidence of RDS **(A)**, intraventricular hemorrhage grades III and IV *(IVH)* **(B)**, sepsis **(C)**, and necrotizing enterocolitis *(NEC)* **(D)** displayed by gestational age at birth.

Table III. Ethnic composition of patients at various tertiary centers

Center	*Ethnicity (%)*				*Total No. of patients*
	Black	*White*	*Hispanic*	*Other*	
University of Alabama					
University Hospital	83.5	16.3	0.0	0.1	3,471
Cooper Green Hospital	61.5	38.2	0.0	0.3	4,676
Subtotal					8,147
University of California, San Diego	4.7	24.4	65.7	5.2	4,280
Vanderbilt University					
Nashville General Hospital	32.8	66.4	0.1	0.6	1,844
Vanderbilt University Hospital	40.7	58.0	0.7	0.7	1,185
Subtotal					3,029
Ohio State University	53.2	44.1	0.1	2.5	2,704
Northwestern University Hospital	43.3	32.5	22.0	2.1	2,520
TOTAL	46.4	35.4	16.3	1.8	20,680

cility, (4) final gestational age between 23 and 44 weeks, (5) birth weight >500 gm, and (6) neonatal survival >24 hours. Infants who died within the first 24 hours of life were excluded, to allow a minimum time for the development and diagnosis of a specific neonatal morbidity, e.g., patent ductus arteriosus, necrotizing enterocolitis.

Gestational age was defined as completed weeks and was analyzed weekly from 23 through 36 weeks and every 2 weeks from 37 until 44 weeks. Thus an infant

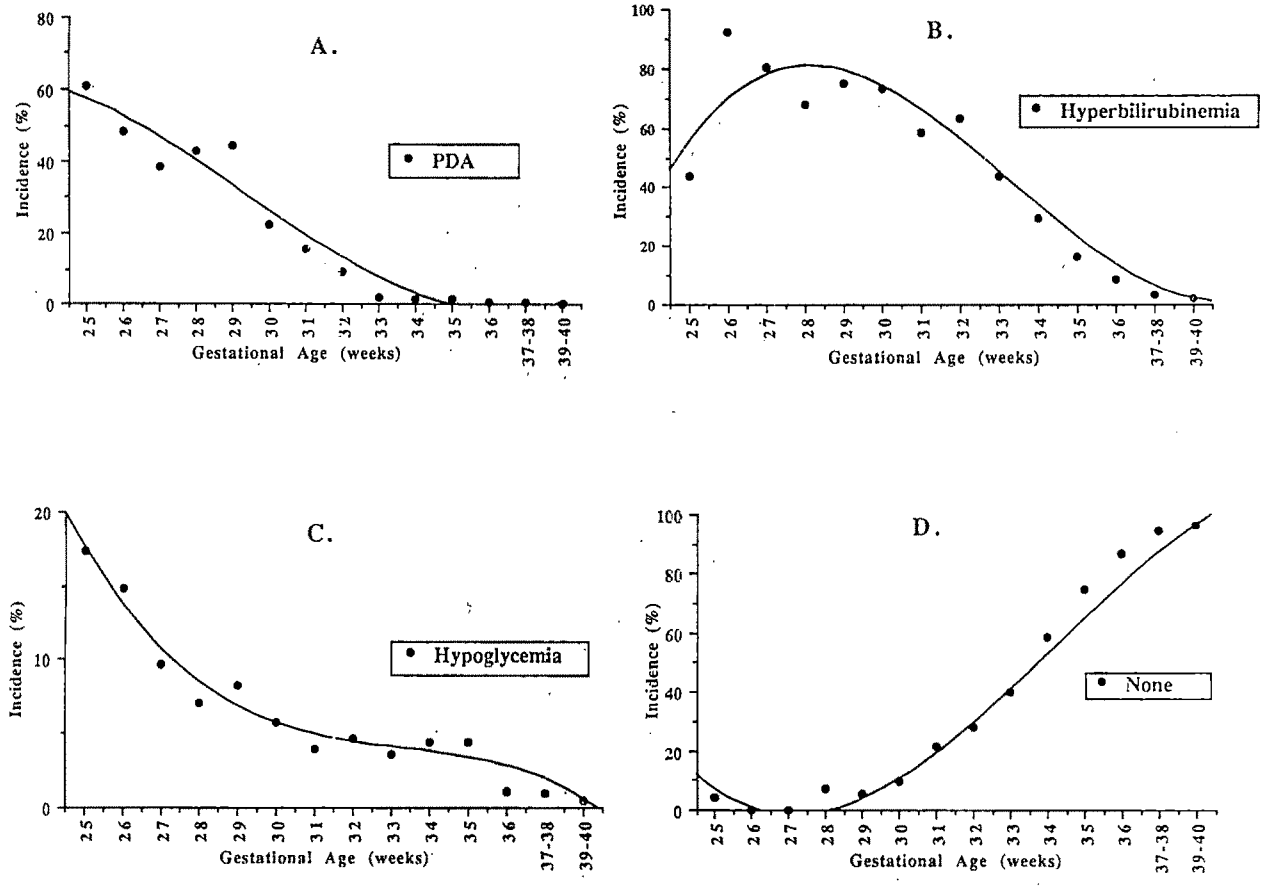


Fig. 2. Incidence of patent ductus arteriosus *(PDA)* **(A)**, hyperbilirubinemia requiring phototherapy **(B)**, hypoglycemia **(C)**, and none of the specific neonatal morbidities **(D)** displayed by gestational age at birth.

delivered at 23⅘ weeks was recorded as 23 weeks of gestation. Birth weight categories were described in 100 gm intervals from 500 until 999 gm, 250 gm intervals from 1000 to 3999 gm, and ≥4000 gm.

Once the subpopulation was defined, additional maternal and neonatal exclusions were based on a maternal or neonatal condition that could affect a specific morbidity or birth weight, e.g., the increased incidence of respiratory distress syndrome (RDS) in neonates of diabetic mothers or the decreased birth weight of those neonates whose mothers have hypertension. Maternal exclusions included mothers with hypertension, preeclampsia, diabetes (classes A through R), abruptio placentae, placenta previa, renal disease, hemoglobinopathies, systemic infections, substance abuse, and collagen disease. Because population-specific growth curves were not available for each geographic region represented, intrauterine growth retardation was not an exclusion. Additionally, the population studied included 46.4% black women; some data suggest that a different population-specific growth curve be used.[3] The data were analyzed before and after the application of the maternal exclusions. Neonatal exclusions included neonates with congenital anomalies (structural or chromosomal) and congenital infections.

Standard definitions of the neonatal morbidity categories were used by the participating institutions. RDS was defined as the early onset of tachypnea, retractions, and oxygen requirement with radiographic confirmation. Infants with transient tachypnea, meconium aspiration, pulmonary edema, and pneumonia were specifically not included in the RDS category. Patent ductus arteriosus was defined as a systolic murmur accompanied by bounding peripheral pulses with either development of pulmonary edema or echocardiographic confirmation of a left-to-right shunt. Intraventricular hemorrhage was diagnosed with routine ultrasonography or computerized tomography and was classified as grade I to IV according to Papile. Infants weighing <1500 gm were routinely screened for intraventricular hemorrhage. Infants weighing ≥1500 gm had radiographic evaluations for intraventricular hemorrhage only if they were symptomatic. Necrotizing enterocolitis was diagnosed by a combination of abdominal disten-

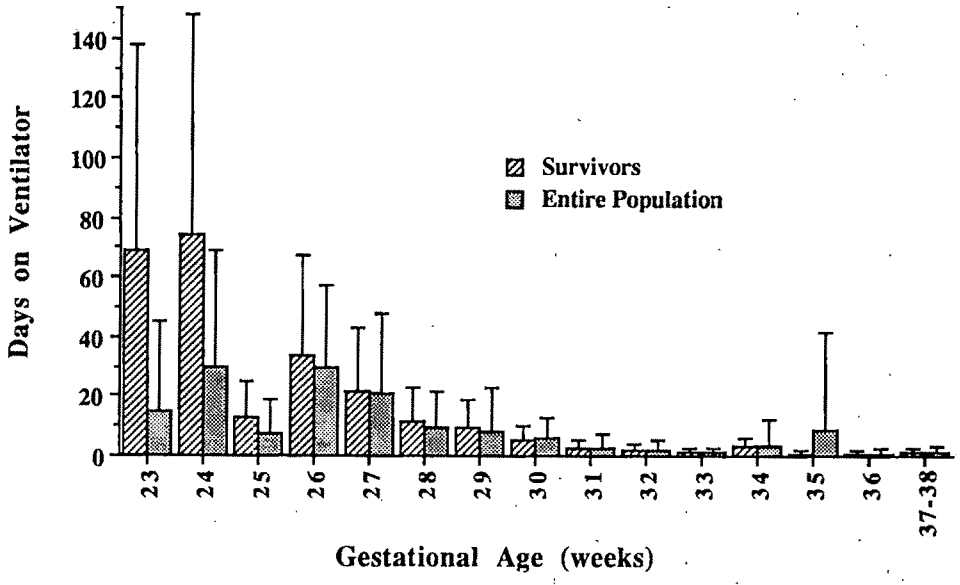


Fig. 3. Mean number of days of mechanical ventilation for all neonates with RDS and survivors with RDS displayed by gestational age at birth.

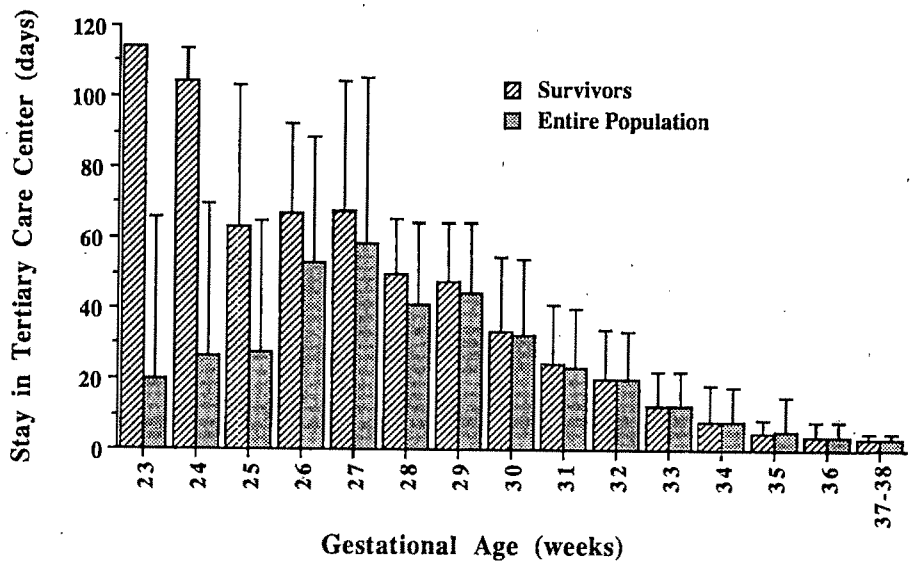


Fig. 4. Mean number of days stay at tertiary care center for total population and survivors, displayed by gestational age at birth.

tion and evidence of intramural air or perforation. Infants with hyperbilirubinemia were described in two groups: those requiring phototherapy and those requiring exchange transfusion. These therapies were initiated according to each nursery's usual protocol. Sepsis was a broad category and included early onset neonatal sepsis or meningitis and nosocomial infections of blood or spinal fluid. Infants with suspected sepsis but negative cultures were not included. Hypoglycemia was defined as blood glucose <30 mg/dl or a capillary qualitative glucose of ≤25 mg/dl.

The incidences of the above-defined neonatal morbidities were calculated by gestational age and birth weight. Similarly, the percent of neonates not having any of the selected morbidities was described. The incidence of admission to the neonatal intensive care unit

Table IV. Incidence (percent) of respiratory distress syndrome by combined gestational age and birth weight

Birth weight (gm)	*Gestational age (wk)*												
	25	*26*	*27*	*28*	*29*	*30*	*31*	*32*	*33*	*34*	*35*	*36*	*37-38*
700-799	100	87											
800-899	100	87	100										
900-999		100	83										
1000-1249			87	73	42								
1250-1499				44	54	68	54	50					
1500-1749					40	47	45	28	48	12	17	25	0
1750-1999						11	17	27	38	18	0	0	0
2000-2249								26	31	11	8	10	2
2250-2499								14	18	17	5	2	1
2500-2749									20	8	7	3	0
2750-2999										23	5	3	0
3000-3249										0	8	0	0
3250-3499												4	0
3500-3749													0
3750-3999													1
≥4000													1
No.	23	27	31	28	36	53	51	107	112	229	298	544	4803

Table V. Incidence (%) of intraventricular hemorrhage (grades III and IV) by combined gestational age and birth weight

Birth weight (gm)	*Gestational age (wk)*												
	25	*26*	*27*	*28*	*29*	*30*	*31*	*32*	*33*	*34*	*35*	*36*	*37-38*
700-799	22	50											
800-899	50	12	50										
900-999		14	0										
1000-1249			7	0	9								
1250-1499				0	0	5	0	0					
1500-1749					0	0	5	2	0	0	0	0	0
1750-1999						0	0	0	0	0	0	0	0
2000-2249								0	0	0	0	0	0
2250-2499								0	0	0	0	0	0
2500-2749									0	0	0	0	0
2750-2999										0	0	0	0
3000-3249										0	0	0	0
3250-3499											0	0	0
3500-3749											0	0	0
3750-3999													0
≥4000													0
No.	23	27	31	28	36	53	51	107	112	229	298	544	4803

or intermediate care unit was also determined The mean number of days on mechanical ventilation for neonates ventilated for RDS and the mean number of days for newborn stay in the tertiary care facility were calculated for both survivors and the entire subpopulation. Because the number of neonates in the gestational age categories 23 and 24 weeks and birth weight categories 500 to 599 and 600 to 699 gm were so small, the incidences of neonatal morbidities in these categories are not included in the figures but are described in Appendixes I and II. For some analyses, the data were further subcategorized by sex and by ethnicity. For selected comparisons in regard to sex or ethnic group, the standard errors of incidence rates were computed to assist in evaluating the significance of observed differences with the formula:

$$\sqrt{\frac{P\,(1-P)}{N}}$$

where N is the total number of neonates and P is the incidence of event (e.g., morbidity). The best-fit fifth-order polynomial curve was plotted to depict the incidence of major neonatal morbidities. Modified birth weight–gestational age charts[4] for gestational ages 25 through 44 weeks and birth weight intervals from 700 to 4000 gm were prepared by plotting incidence rates by combined gestational age and birth weight for a more specific examination of how a birth weight cate-

Gestational age (wk)			
39-40	41-42	43-44	No.
			31
			25
			21
			58
			77
0			130
0			160
5	0		377
0	0		763
0	1	0	1626
0	1	0	2808
0	0	5	3982
0	1	0	4076
0	0	1	3159
1	0	0	1808
0	1	2	1560
9981	3955	384	

Gestational age (wk)			
39-40	41-42	43-44	No.
			31
			25
			21
			58
			77
0			130
0			160
2			377
0	0		763
0	0	0	1626
0	0	0	2808
0	0	0	3982
0	0	0	4076
0	0	0	3159
0	0	0	1808
0	0	0	1560
9981	3955	384	

gory affected morbidity at a certain gestational age or vice versa. Only those combined cells with four or more neonates are reported. These combined charts were prepared for RDS, intraventricular hemorrhage, sepsis, necrotizing enterocolitis, patent ductus arteriosus, and hypoglycemia.

Results

A total of 25,481 babies were delivered at the five centers and are recorded as part of our study population. Table I describes the various exclusions resulting in a final population of 20,680 neonates. Conditions contributing to maternal exclusions are listed in Table II. Table III describes the ethnicity of the pregnant women by center. The overall ethnic composition was 46.4% black, 35.4% white, 16.3% Hispanic, and 1.8% other. Table III also includes the total number of patients contributed by each of the seven hospitals in the five different centers. Maternal age distribution was fairly even, with ages 21 through 25 years contributing the largest number of patients to the study. Fifty percent of the patients were married. Educational level varied: 59.4% of the population had ≥12 years of education and 11.8% had ≤8 years. Sixty-eight percent of the population had seven or more prenatal visits, 15% had four to six visits, 9% had one to three visits, and 8% had no prenatal visits. Evaluation of data accuracy by reabstraction of data by the nurse-coordinator revealed an error rate of <1%. These errors were random in occurrence. Only 4.2% of the 20,680 neonates in our subpopulation had a final gestational age that required adjustment by the independent observer.

We would like to emphasize that there were differences in management protocols between the five centers with respect to the administration of corticosteroids for fetal lung maturation, the use of tocolytics, and the management of prolonged premature rupture of membranes. Of the 697 deliveries occurring from the twenty-third through the thirty-fourth week of gestation, 195 (30%) had ruptured membranes at the time of admission. Of the 502 with intact membranes, 358 (71%) received parenteral or oral tocolytic medications and 101 (20%) received corticosteroids for fetal lung maturation. Because our priority was to study the incidence of overall neonatal morbidities in uncomplicated pregnancies and to obtain the largest number of neonates per gestational age category, we did not subdivide the already small number of neonates into groups on the basis of membrane status or medications received.

Figs. 1 and 2 describe the incidence of specific neonatal morbidities from 25 weeks of gestation to term. Data from the twenty-third and twenty-fourth week were not included because the number of neonates in each category were so small (6 and 12, respectively). Fig. 1 includes data on the gestational age–specific incidence of RDS *(A)*, grade III and IV intraventricular hemorrhage *(B)*, sepsis *(C)*, and necrotizing enterocolitis *(D)*. Fig. 2 depicts similar data for patent ductus arteriosus *(A)*, hyperbilirubinemia treated with phototherapy *(B)*, and hypoglycemia *(C)*. Fig. 2, *D*, describes the gestational age–specific incidence of having neither hyperbilirubinemia treated by exchange transfusion nor one or more of the other six specific morbidities. Fig. 3 describes the mean number of days (with standard deviation) of mechanical ventilation for neonates in whom RDS developed. Data for both surviving infants and all infants ventilated are presented. In the 35-week category, there is an increase in the mean num-

Table VI. Incidence (percent) of sepsis by combined gestational age and birth weight

Birth weight (gm)	Gestational age (wk)												
	25	26	27	28	29	30	31	32	33	34	35	36	37-38
700-799													
800-899													
900-999													
1000-1249			20	20	27								
1250-1499					15	14	15						
1500-1749						7	10	0	5				
1750-1999							17	4	3	7	0		0
2000-2249								4	12	3	6	0	0
2250-2499									0	2	1	0	1
2500-2749										3	4	3	0
2750-2999										0	0	0	1
3000-3249											0	0	0
3250-3499												0	0
3500-3749													0
3750-3999													0
≥4000													
No.	23	27	31	28	36	53	51	107	112	229	298	544	4803

Table VII. Incidence (percent) of necrotizing enterocolitis by combined gestational age and birth weight

Birth weight (gm)	Gestational age (wk)												
	25	26	27	28	29	30	31	32	33	34	35	36	37-38
700-799	11	12											
800-899	22	12	0										
900-999		14	0										
1000-1249			13	27	25								
1250-1499				11	8	18	8	13					
1500-1749					0	13	5	7	0	12	0	0	0
1750-1999						0	8	5	3	4	0	11	0
2000-2249								4	0	3	0	0	0
2250-2499								0	0	0	0	1	0
2500-2749									0	3	0	2	0
2750-2999										0	3	0	0
3000-3249										0	0	0	0
3250-3499												0	0
3500-3749												0	0
3750-3999													0
≥4000													0
No.	23	27	31	28	36	53	51	107	112	229	298	544	4803

ber of days ventilated at 34 weeks from 3.1 to 8.7. However, one neonate is responsible for this increase (birth weight of 750 gm at 35 weeks with 147 days on mechanical ventilation). If this one neonate was eliminated, mean number of days would become 1.0 ± 1.53 days. Fig. 4 describes the mean number of days (with standard deviation) of neonatal stay in the tertiary care facility for both all neonates and survivors.

All morbidities were also examined in subcategories of ethnicity, maternal age, and newborn sex. No statistically significant differences were found except in two subgroups. The incidence of hypoglycemia was significantly higher in Hispanic neonates weighing 4000 to 4999 gm (2.8% in Hispanic, 1.3% in white, and 1.5% in black neonates), and the incidence of neonatal hypoglycemia was higher for women over the age of 30 (0.8% <30 vs 2.1% >35). This latter finding occurred despite the exclusion of maternal diabetes.

Tables IV, V, VI, VII, VIII, and IX describe the incidence of the more frequent major neonatal morbidities (RDS, intraventricular hemorrhage, sepsis, necrotizing enterocolitis, patent ductus arteriosus, and hypoglycemia) within combined birth weight–gestational age categories.

Appendix I contains a complete tabulation on all data on the specific neonatal morbidities and the frequency

Gestational age (wk)			
39-40	41-42	43-44	No.
			31
			25
			21
			58
			77
			130
			160
0			377
0	0		763
0	0	0	1626
0	0	0	2808
0	0	0	3982
0	1	0	4076
1	0	1	3159
1	0	0	1808
0	1		1560
9981	3955	384	

Gestational age (wk)			
39-40	41-42	43-44	No.
			31
			25
			21
			58
			77
0			130
0			160
0	0		377
1	0		763
0	0	0	1626
0	0	0	2808
0	0	0	3982
0	0	0	4076
0	0	0	3159
0	0	0	1808
0	0	0	1560
9981	3955	384	

of admission to the intensive care nursery displayed by gestational age. Appendix II includes the same data displayed by birth weight category.

Comment

This study presents data from a large number of pregnant women who had prospectively dated pregnancies and whose neonates were examined for specific morbidities. Pregnancies from five centers across the United States are included. The only previous study of neonatal morbidity in well-dated pregnancies was in a very limited population of 170 neonates.[5] Maternal demographic characteristics of our population generally represent low- to middle-class women with a large representation by blacks. The majority of the pregnant women (68.7%) in this study had more than six prenatal visits. The combination of the large number of prenatal visits by the majority of women and the readjustment of only 4.2% of final gestational ages supports the accuracy of prenatal assignment of gestational age.

The incidence of RDS is probably the most frequently studied morbidity published in the literature. Our study confirms previous studies of the incidence at approximately a 55% to 65% risk at 28 to 30 weeks of gestation and 30% to 40% risk at 31 to 33 weeks of gestation. Our study also shows an extremely high incidence (80% to 90%) of RDS from 25 to 27 weeks of gestation. These incidences are similar to the smaller study by Konte et al.[5] However, one difference from that study is the increased incidence of RDS at 35 (6.4%) and 36 weeks of gestation (3.3%). This incidence at 35 and 36 weeks could possibly be decreased by more aggressive use of amniocentesis to identify the fetus at risk and use of tocolysis and corticosteroids as indicated. A report in 1979 by Ballard et al.[6] showed a 93% incidence of RDS in infants 500 to 750 gm whose mothers did not receive antenatal steroids. This figure is slightly higher than our incidence in birth weight category of 500 to 799 gm. More recent publications compare the incidence of RDS in control versus treatment groups with various modalities including antenatal steroids, thyroid-releasing hormone, tocolytics, and prophylactic surfactant replacement.[7-9] Our data consists of infants born to mothers in tertiary centers, some of whom had prolonged ruptured membranes, antenatal steroid therapy, or tocolytic therapy and should reflect optimal management at that time. Still, the incidence of RDS in our study is extremely high at 23 to 27 weeks of gestation. Because the severity of RDS was not coded on the data entry form, the cases of severe versus moderate or mild disease could not be delineated. Our data fail to confirm a consistently decreased incidence of RDS in black neonates compared with white neonates when analyzed by gestational age or birth weight. Of note is the decrease in the incidence with increasing birth weight within each gestational age category between 27 to 32 weeks and the decrease in incidence with increasing gestational age within each birth weight category between 1500 and 3000 gm (Table IV). Surprisingly, within the various gestational age categories at 35 to 38 weeks, there is no increase in the incidence as birth weight increases. Perhaps this finding is related to the exclusion of infants of diabetic mothers from this analysis.

The mean number of days on mechanical ventilation for the entire population with RDS (Fig. 3) does not consistently decrease with advancing gestational age

Table VIII. Incidence (percent) of patent ductus arterisous by combined gestational age and birth weight

Birth weight (gm)	*Gestational age (wk)*												
	25	*26*	*27*	*28*	*29*	*30*	*31*	*32*	*33*	*34*	*35*	*36*	*37-38*
700-799	78	37											
800-899	44	62	75										
900-999		43	33										
1000-1249			20	47	50								
1250-1499				33	31	27	31	12					
1500-1749					40	13	10	12	9	12	0	0	0
1750-1999						33	17	9	0	4	8	0	0
2000-2249								4	0	1	0	0	0
2250-2499								0	0	0	1	0	0
2500-2749									0	0	0	0	0
2750-2999										6	0	1	0
3000-3249										0	0	0	0
3250-3499												0	0
3500-3749												0	0
3750-3999													1
≥4000													0
No.	23	27	31	28	36	53	51	107	112	229	298	544	4803

Table IX. Incidence (percent) of hypoglycemia by combined gestational age and birth weight

Birth weight (gm)	*Gestational age (wk)*												
	25	*26*	*27*	*28*	*29*	*30*	*31*	*32*	*33*	*34*	*35*	*36*	*37-38*
700-799	22	25	33										
800-899	11	25	25										
900-999		0	17										
1000-1249			0	0	8								
1250-1499				11	15	4	0	0					
1500-1749					0	7	10	7	5	25	0	0	0
1750-1999							0	0	3	0	8	0	6
2000-2249								0	0	5	2	2	4
2250-2499								0	0	4	6	0	1
2500-2749									40	3	2	1	1
2750-2999										0	3	1	1
3000-3249										0	8	2	1
3250-3499												0	1
3500-3749												0	0
3750-3999													3
≥4000													3
No.	23	27	31	28	36	53	51	107	112	229	298	544	4803

until 26 weeks of gestation, reflecting the mortality of those neonates with RDS at 23 to 25 weeks. For those survivors at 23 to 25 weeks with RDS, the average number of days on the ventilator peaks at 24 weeks (74 days) and gradually decreases with increasing gestational age. It is of note that there is an increase in mechanical ventilation required for neonates at 34 weeks of gestation, reflecting the presence of RDS as a morbidity at 34 weeks of gestation.

In this report we have focused on the incidence of severe intraventricular hemorrhage (grades III and IV), which has been associated with a definite increase in long-term morbidity, developmental delay, and neurologic abnormalities. Our data on the incidence of severe bleeding are accurate up to 1500 gm. However, we may have missed some instances in larger babies who were not evaluated radiographically because they were asymptomatic. Nonetheless, the incidence of symptomatic severe bleeding drops to zero after 32 completed weeks of gestation. In Konte et al.'s[5] small study the incidence was higher in the category 28 to 33 weeks than in our findings, but their numbers were much smaller than those of our larger study ($n = 33$ vs our 104 at 30 to 31 weeks). The incidence of intracranial hemorrhage in the literature, usually by birth weight groups, has been reported as 40% at <1500 gm.[10] McGuiness and Smith[11] reported on 112 consecutive babies between 1500 and 2000 gm who were all asymptomatic but had a 13% incidence intraventricular hemorrhage. Shinnar et al.[12] reported the incidence of

Gestational age (wk)			
39-40	41-42	43-44	No.
			31
			25
			21
			58
			77
0			130
0			160
0			377
0	0		763
0	0	0	1626
0	0	0	2808
0	0	0	3982
0	0	0	4076
0	0	0	3159
0	0	0	1808
1	1	1	1560
9981	3955	384	

Gestational age (wk)			
39-40	41-42	43-44	No.
			31
			25
			21
			58
			77
20			130
0			160
0	0		377
2	0		763
0	1	0	1626
0	0	0	2808
0	0	0	3982
0	0	0	4076
0	0	0	3159
0	0	0	1808
2	2	3	1560
9981	3955	384	

various grades of intraventricular hemorrhage by gestational age in infants weighing <1800 gm. At <26 weeks of gestation, two had grade III and IV hemorrhages, whereas by 33 to 36 weeks, none had grade III or IV hemorrhages. Pooled data on infants <1500 gm from six centers reported by Allan and Philip[13] showed the incidence of any grade of intraventricular hemorrhage to be 77% at <26 weeks of gestation, 56% at 27 to 28 weeks of gestation, 37% at 29 to 30 weeks of gestation, and 7% at or beyond 33 weeks.

In general, the incidence of patent ductus arteriosus in our study is lower than the incidence previously reported in the literature. In 1972, Kitterman et al.[14] reported a dramatically increased incidence in preterm infants. In that study in 15.3% of infants weighing <1750 gm a hemodynamically significant ductus shunt developed. Heymann[15] in 1977 reported a 45% incidence in infants weighing <1750 gm with an incidence as high as 75% to 80% in infants <1250 gm. In 1989 this very high incidence in extremely low-birth-weight infants was confirmed by Cassady et al.,[16] who found an incidence of 81% in infants weighing <1000 gm and in oxygen for lung disease. Gersony et al.,[17] in the large collaborative study of indomethacin, reported an incidence of 21% in infants weighing <1750 gm. A study of the incidence of patent ductus arteriosus on the basis of gestational age was performed by Siassi et al.[18] in 1976. The risk was as follows: 17% at 28 to 30 weeks, 44% at 34 to 36 weeks, and 7.7% at 37 to 40 weeks. This high incidence at term may represent some infants with congenital heart disease. More recently, John and Todd[19] in Australia in 1984 and 1985 evaluated at babies at 24 to 26 weeks of gestation and found significant patent ductus arteriosus in only 15 of 34 or 44%. The lower incidence in his study and in our collaborative data may reflect changes in antenatal management, including the administration of betamethasone. In fact, in 1981 Clyman et al.[20] described a significant decrease in the incidence of patent ductus arteriosus after maternal betamethasone therapy in infants weighing 500 to 1500 gm. In our gestational age–birth weight data (Table VIII), the incidence of patent ductus arteriosus decreases with advancing gestational age in the same birth weight category and also decreases with increasing birth weight within the same gestational age category. This finding parallels the pattern of the combined chart on birth weight and gestational age for RDS.

The incidence of necrotizing enterocolitis is quite variable in our study but is high up to 31 weeks of gestation, when it begins to decline, becoming quite low after 34 weeks of gestation. The incidence in the literature has varied from 3% to 10% in infants weighing <1500 gm and remains at about 1% until term.[21] The gestational age–birth weight data on necrotizing enterocolitis (Table VII) did not reveal a definite pattern of decreasing incidence until 35 weeks of gestation, or of birth weight >2000 gm, when the occurrence became rare.

The incidence of neonatal sepsis in our study was very high up to 29 weeks of gestation (25%-35%) and declined by 32 weeks. It then remained stable at 1% to 5% until a further drop at 37 weeks to 0.3% to 0.5%. The incidence of neonatal sepsis in all live births has been reported as between 0.1% to 0.4% over the last decade.[22] The incidence of infections seen in neonatal intensive care units has been much higher and continues to rise with an increasing incidence of nosocomial infections in very immature neonates with prolonged and complicated hospitalizations. Recent studies in infants weighing <1500 gm have reported incidences

varying from 8% to 70%. The National Institutes of Health neonatal network collaborative intravenous immunoglobulin trial reported a 33% incidence between 500 and 750 gm, dropping to 10% for 1250 to 1500 gm neonates.[23] Our gestational age–birth weight data on sepsis (Table VI) were similar to the above chart on necrotizing enterocolitis until 35 weeks or >2000 gm when sepsis was at a baseline of 0% to 3% with no definite pattern.

The incidence of hypoglycemia in our study was definitely higher in the very immature neonates (24 to 26 weeks of gestation) and then declined as gestational age advanced. However, in the data arranged by birth weight there is an increase in hypoglycemia in infants weighing ≥4000 gm, although infants of diabetic women and diabetic pregnant women have been excluded from our study. It is noteworthy that all centers were not routinely screening for gestational diabetes in all pregnant women during the years of the data collection. Additionally, most babies were not routinely checked for hypoglycemia. Therefore it is possible that the low incidence of hypoglycemia in term, average-sized newborns possibly reflects the fact that screening did not occur. The higher values in the small and large infants may well represent an ascertainment bias. When the birth-weight category of 4000 to 4999 gm was scrutinized carefully by ethnic group, Hispanic neonates were overrepresented, with a 2.8% incidence of neonatal hypoglycemia. This finding supports previous findings that Hispanic pregnant women have a higher incidence of gestational diabetes than white or black women.[24]

Data on mean length of stay for neonates in tertiary care facilities (Fig. 4) may be of interest to administrators in considering allocation of resources. Obviously, these data are influenced by the availability and willingness of community-based nurseries to accept transfer of these neonates. The mean length of stay was 3 days for babies at least 37 weeks of gestation but increased steadily with decreasing gestational age to a peak mean stay of 58 days at 27 weeks. At <27 weeks of gestation, infants had shorter stays, presumably because of increased mortality after the first days of life. However, when parents are counseled, the table of length of stay for survivors conveys much more accurately the likely duration of hospitalization if the infant survives the early neonatal course.

Virtually all infants <33 weeks of gestation were admitted to the intermediate or intensive care nursery. At term 10% of infants were admitted. The admission rate rose to 14% at 43 to 44 weeks and reflects the increased morbidity of postterm gestation.

We were concerned by the relatively small number of infants delivered between 23 and 34 weeks of gestation and wondered about the wisdom of our maternal exclusions. We considered the effect of these exclusions by comparing the incidence of each specific morbidity in the various weight and gestational age categories for the excluded neonates (3004) and our study populations. If the exclusions had not been made, there would have been statistically significant differences in 25 of 144 birth-weight categories and 16 of 144 gestational age categories. If we added the excluded patients the effect would be to increase the incidence of the various morbidities at the lower gestational ages and birth weights and decrease it in the large and more mature neonates.

Comment

The data presented represent the largest series of accurately dated pregnancies in which the incidence of neonatal morbidities is studied by gestational age and birth weight. In general, the incidence of both RDS and patent ductus arteriosus is markedly decreased by both increasing gestational age and birth weight. The incidence of RDS is still significant at 35 to 36 weeks of gestation. The incidence of grade III and IV intraventricular hemorrhage decreases to zero after 32 completed weeks of gestation. The incidence of necrotizing enterocolitis declines after 31 weeks of gestation and is very low after 34 weeks of gestation. The incidence of neonatal sepsis decreases after 32 weeks of gestation. The incidence of neonatal hypoglycemia is increased in premature neonates and declines gradually with increasing gestational age, unless the birth weight is ≥4000 gm, when the incidence is increased at 1.9%. This information about the incidence of neonatal morbidities by gestational age should be invaluable to the obstetrician in the critical decision regarding the timing of the delivery of the fetus and to the parents who can benefit from a realistic prediction of the neonatal course.

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Discussion

DR. JIMMIE WESTBERG, San Francisco, California. Dr. Laros has presented an excellent paper describing a large series of obstetric patients who had meticulous, prospective gestational dating confirmed by Dubowitz examination of the respective newborns.

Selective morbidities of the newborn were evaluated both by the accurate gestational dates and birth weights. The purpose of this evaluation was to establish incidence norms for the specific morbidities to help in the complex decision process regarding preterm birth. However, maturity-related problems and infections are not the only problems faced in making these decisions. Neonatal mortality, neurologic handicaps, and learning disabilities must also be considered. This article accomplished its goals in a limited way. It is a beginning. It is unfortunate that mortality data were not specifically identified, as clearly these data were available.

Having been involved in the California Medical Association's Neonatal Mortality Studies for California's Health Services,[1] I can appreciate the unbelievable time and effort that goes into such studies. Devising the forms, overseeing their completion, and reviewing the computer printouts requires superhuman work, particularly when seven different hospitals are involved.

I would now like to discuss portions of this study by comparing results to the California Neonatal Mortality Study for 1984.

The present study involved tertiary centers throughout the United States. In California we have fairly well-defined obstetric units called regional centers and neonatal units noted as California Children's Services, levels I, II, and III. Usually regional centers are associated with level III nurseries. Table I shows the total live births in the designated neonatal units and their respective neonatal death rates. Only 18% of total births occurred in California's tertiary centers, certainly not a representative sampling. It is interesting to note that 31% of the deaths in level III nurseries were thought to be preventative because of suboptimal care rendered by these centers.

Table II compares the racial breakdown of the two studies listing the California State mortality data and Dr. Laros' morbidity data. The disparity between the two studies is obvious. Because mortality and morbidity rates usually parallel each other, it is apparent from this table that the black neonatal mortality rate is significantly different from all other groups. The morbidity study showed no such differences. Table III, which shows data from the California mortality study,

Table I. Place of delivery by preventability, in-hospital neonatal deaths, California, 1984

California Children's Services level of delivery hospital preventability	*Frequency and percentage*	*Total live births**	*Neonatal death rate/1000 live births†*
Home	3 (1.1%)	4,825	6.0
Preventable	2 (66.7%)		
Level I	113 (42.3%)	283,108	3.9
Preventable	35 (31.0%)		
Level II	76 (28.5%)	78,167	9.4
Preventable	28 (36.8%)		
Level III	74 (27.7%)	80,015	9.0
Preventable	23 (31.1%)		
TOTAL	267 (100%)	447,351	6.0

*According to the State of California, Department of Health Services, 1984 Birth Cohort.

†Frequencies are extrapolated from the 10.3% random sample to the total number of deaths in California to calculate rates.

Table II. Race of mother, in-hospital neonatal deaths, California, 1984

Race of mother	*Live Births (no. and %)*	*Race-specific neonatal death rate/1000 live births*	*Proportion of Morbidity study (%)*
White	229,053 (51.2%)	4.9	35.4
Hispanic	133,023 (29.7%)	5.0	16.3
Black	38,639 (8.6%)	13.1	46.4
Other	44,216 (9.9%)	5.7	1.8
No information	2,394 (0.5%)	—	
TOTAL	447,325 (100%)	—	100% (20,680)

may provide a partial answer to this racial discrepancy. Approximately 20% of all neonatal deaths occurred in <500 gm group. If only the black neonates are considered, 29% were <500 gm and 31% were between 500 and 750 gm. Most of these infants would have been excluded from the morbidity study. This is further substantiated by the fact that the median birth weight of black deceased neonates was 585 gm.

Several other points in this article are disturbing to me. Eight percent of the included mothers had no prenatal care yet were meticulously dated. Pregnancies with preterm labor and delivery are not uncomplicated pregnancies. A 750 gm infant born at 35 weeks who is like a 27-week neonate is not compatible with meticulous dating.

In conclusion, this is an excellent beginning. However, this study covers only a small section of our obstetric delivery system, has a distorted racial mix of patients, and has too many exclusions. In the future I hope studies of neonatal mortality and morbidity will be undertaken with standardized obstetric and neonatal forms that are meticulously completed. With this information accurate mortality and morbidity rates will be ascertained for each racial group. For now, we can say to our patients, "If your baby is born after more than 23 weeks and lives more than 24 hours, we can make some projections as to morbidity, duration of hospitalization, and financial cost."

I have several questions for Dr. Laros: (1) Why were patients from the University of California, San Francisco not included? (2) What percentage of the births occur in the tertiary centers compared with their respective catchment areas? (3) How were the meticulous dating criteria applied to the pregnancies of mothers with no prenatal care? and (4) Was the 750 gm infant who was born at 35 weeks small for gestational age?

REFERENCE

1. California Medical Association In-Hospital Neonatal and Fetal Mortality Study. Maternal and Child Health Branch, California Department of Health Services: 1984.

DR. ROBERT C. GOODLIN, Placerville, California. The March of Dimes spent millions of dollars and, until now, we have heard no results. In my opinion this has been a real "black eye" for academic obstetrics. As far as I know, this is the first detailed analysis of those data. If Dr. Laros' study can be done, I don't understand why we haven't heard the rest of the story. My suspicion is, of course, that there was a negative outcome.

I have two other questions that concern RDS. First, I have always believed there was an increased incidence between 33 and 35 weeks and I thought I saw a little peak on Fig. 3. Did you see it? Second, I have also thought that when RDS occurs after 34 weeks it is often a more severe disease process. Do your data support this concept?

DR. JULIAN T. PARER, San Francisco, California. I have two comments. First, I was much more comfortable with the conclusions you previously reported—that 34 weeks was the time when the cost/benefit ratio shifted so that financially it was better to deliver a baby

Table III. Race of mother by birth weight, in-hospital neonatal deaths, California, 1984

Birth weight (gm)	*Race of mother (No. and %)*					
	White	*Hispanic*	*Black*	*Other*	*No information*	*Total*
0-499	24 (20.9%)	14 (20.3%)	15 (28.8%)	1 (3.8%)	1 (20.0%)	55 (20.6%)
500-749	19 (16.5%)	10 (14.5%)	16 (30.8%)	7 (26.9%)	1 (20.0%)	53 (19.8%)
750-999	16 (13.9%)	7 (10.1%)	5 (9.6%)	3 (11.5%)	2 (40.0%)	33 (12.4%)
1000-1499	12 (10.4%)	8 (11.6%)	1 (1.9%)	2 (7.7%)	0 (0.0%)	23 (8.6%)
1500-2499	24 (20.9%)	12 (17.4%)	6 (11.5%)	6 (23.1%)	1 (20.0%)	49 (18.4%)
2500-3999	16 (13.9%)	14 (20.3%)	7 (13.5%)	5 (19.2%)	0 (0.0%)	42 (15.7%)
≥4000	3 (2.6%)	2 (2.9%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	5 (1.9%)
Unknown	1 (0.9%)	2 (2.9%)	2 (3.8%)	2 (7.7%)	0 (0.0%)	7 (2.6%)
TOTAL	115 (100%)	69 (100%)	52 (100%)	26 (100%)	5 (100%)	267 (100%)

Note: Numbers are separated by percentages in each racial category. The median birth weight for deceased neonates of white mothers was 905 gm; Hispanic mothers, 1080 gm; black mothers, 585 gm; and mothers of other races, 1345 gm.

rather than keep the mother in the hospital generating a large bill.[1] I am now a little uncomfortable with that conclusion because you have clearly shown a continuum of morbidity. Where on that continuum of newborn morbidity after 34 weeks would you allow a woman to be delivered? That is, what is an acceptable degree of morbidity?

My second question regards doing amniocentesis to determine pulmonary maturity after 34 weeks, when betamethasone has not been shown to be effective. It seems to me that because the incidence of respiratory distress syndrome is going to be 5%, or certainly in the single-digit percentages, most of those amniocenteses, lecithin/sphingomyelin ratios, phosphatidylglycerol determinations, and much of the betamethasone treatment might well be largely wasted. The question can and should be studied from the standpoint both of the cost/benefit ratio and of morbidity before we accept amniocentesis as the preferred procedure at that stage.

REFERENCE

1. Korenbrot CC, Aalto LH, Laros RK Jr. The cost-effectiveness of stopping preterm labor with beta-adrenergic treatment. N Engl J Med 1984;310:691.

Dr. Laros (Closing). Dr. Westberg points out very clearly a dilemma that we faced. When the data analysis for the State of California is done, birth and death certificate information is used. Although this provides a large number of patients, the data are flawed by inaccuracies in the gestational age and by the limited number of diagnoses available on the records of both mother and infant. On birth certificates and fetal death certificates there are only four boxes for diagnosis, and we all know that many neonates who have been in the intensive care nursery have many important problems. These problems simply are not included in the data set. In our study, on the other hand, we have very precise data on a limited number of patients. As expensive as collecting these data was, to go further and attempt to gather information on learning disabilities of the offspring would be prohibitively expensive.

Why was the University of California, San Francisco not a study site? Our department already had an educational program and thus would not have been an appropriate study site.

We do not know what percentage of the total population in the area was represented by the infants delivered in each of these tertiary centers. It would be interesting to know how representative our sample was.

With respect to the meticulous dating on patients with no prenatal care, this 8% of our population was dated by the Dubowitz score, the best criterion available for patients with no prenatal care.

I do not believe that we had a 750 gm infant delivered at 35 weeks in our data set. If we had one, it obviously would have been an example of marked intrauterine growth retardation.

Dr. Goodlin, for political reasons, I will not answer the question of why so few data have been made public from the March of Dimes study. In the immediate future you can expect to see a fairly large number of publications by various investigators using this data set. The involved principal investigators agreed that it was most appropriate to separate into three reports the gestational age and birth weight data, the mortality data, and the morbidity data. Dr. Westberg, our article is lengthy, with a number of tables and appendixes, and the companion articles will have similar amounts of data.

Dr. Parer, the analysis that we have previously published[1] says that at 34, 35, and 36 weeks it is most cost-effective to allow a mother to be delivered. These data say that at these gestational ages there is a very low incidence of RDS, sepsis, and hyperbilirubinemia. Patent ductus arteriosus and intraventricular hemorrhage do not occur. You inspire me to do a cost-effectiveness analysis of amniocentesis and betamethasone use during the thirty-fourth and thirty-fifth weeks.

REFERENCE

1. Korenbrot CC, Aalto LH, Laros RK Jr. The cost-effectiveness of stopping preterm labor with beta-adrenergic treatment. N Engl J Med 1984;310:691.

Appendix I. Neonatal morbidity by gestational age

Morbidity	Gestational age (wk)						
	23	24	25	26	27	28	29
Respiratory distress syndrome	83.3	66.7	87.0	92.6	83.9	64.3	52.8
Intraventricular hemorrhage (grades III and IV)	16.7	25.0	30.4	29.6	16.1	3.6	2.8
Sepsis	33.3	25.0	8.7	33.3	35.5	25.0	25.0
Necrotizing enterocolitis	16.7	8.3	17.4	11.1	9.7	25.0	13.9
Patent ductus arteriosus	16.7	33.3	60.9	48.1	38.7	42.9	44.4
Phototherapy for hyperbilirubinemia	16.7	66.7	43.5	92.6	80.6	67.9	75.0
Exchange transfusion for hyperbilirubinemia	0.0	0.0	04.3	03.7	06.5	0.0	02.8
Hypoglycemia	0.0	8.3	17.4	14.8	9.7	7.1	8.3
None of the above	16.7	16.7	4.3	.0	.0	7.1	5.6
Admission to intensive care or special care nursery	100.0	100.0	100.0	100.0	100.0	100.0	100.0
TOTAL (N = 20,680)	6	12	23	27	31	28	36

Appendix II. Neonatal morbidity by birth weight

Morbidity	Birth weight (gm)							
	500-599	600-699	700-799	800-899	900-999	1000-1249	1250-1499	1500-1749
Respiratory distress syndrome	85.7	58.3	77.4	96.0	85.7	65.5	51.9	32.3
Intraventricular hemorrhage (grades III and IV)	14.3	25.0	29.0	28.0	9.5	6.9	1.3	1.5
Sepsis	14.3	33.3	16.1	20.0	47.6	20.7	15.6	3.8
Necrotizing enterocolitis	28.6	8.3	12.9	16.0	19.0	20.7	13.0	5.4
Patent ductus arteriosus	42.9	41.7	48.4	56.0	42.9	31.0	24.7	10.8
Phototherapy for hyperbilirubinemia	28.6	66.7	71.0	76.0	90.5	69.0	70.1	61.5
Exchange transfusion for hyperbilirubinemia	0.0	0.0	12.9	4.0	0.0	0.0	0.0	0.8
Hypoglycemia	14.3	16.7	16.1	16.0	14.3	5.2	6.5	7.7
None of the above	14.3	8.3	6.5	0.0	0.0	0.0	11.7	26.2
Admission to intensive care unit	100.0	100.0	100.0	100.0	100.0	100.0	96.1	99.2
Total (N = 20,680)	7	12	31	25	21	58	77	130

Gestational age (wk)										
30	*31*	*32*	*33*	*34*	*35*	*36*	*37-38*	*39-40*	*41-42*	*43-44*
54.7	37.3	28.0	33.9	13.5	6.4	3.3	0.4	0.3	0.5	1.6
1.9	2.0	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
11.3	13.7	2.8	5.4	3.5	2.3	1.3	0.3	0.4	0.4	0.5
15.1	7.8	5.6	1.8	3.1	0.3	0.9	0.0	0.1	0.0	0.0
22.6	15.7	9.3	1.8	1.7	1.3	0.4	0.3	0.2	0.4	1.0
73.6	58.8	63.6	43.8	29.3	16.4	9.0	3.5	2.3	2.7	2.3
0.0	0.0	0.9	0.0	0.9	0.3	0.4	0.2	0.1	0.2	0.0
5.7	3.9	4.7	3.6	4.4	4.4	1.1	0.9	0.5	0.5	0.8
9.4	21.6	28.0	40.2	58.5	74.8	86.6	94.8	96.5	95.5	94.8
94.3	96.1	98.1	83.9	70.3	41.6	24.1	10.2	9.6	12.6	16.9
53	51	107	112	229	298	544	4803	9981	3955	384

Birth weight (gm)									
1750-1999	*2000-2249*	*2250-2499*	*2500-2749*	*2750-2999*	*3000-3249*	*3250-3499*	*3500-3749*	*3750-3999*	*>4000*
16.9	10.1	3.0	1.2	0.6	0.4	0.4	0.3	0.5	0.6
0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5.6	2.9	0.7	0.7	0.4	0.3	0.4	0.5	0.5	0.5
3.1	0.8	0.3	0.2	0.0	0.1	0.0	0.1	0.0	0.0
6.3	0.8	0.3	0.0	0.3	0.2	0.3	0.2	0.6	0.8
39.4	16.4	8.8	4.8	3.0	.3	2.6	2.4	1.9	2.9
0.0	0.3	0.3	0.2	0.2	0.1	0.1	0.2	0.1	0.2
2.5	2.7	2.0	1.0	0.5	0.5	0.4	0.3	0.6	1.9
46.9	76.4	87.0	93.3	95.7	95.8	96.1	96.4	96.1	93.5
83.8	48.3	24.9	15.8	9.7	9.2	10.5	9.6	10.2	14.3
160	377	763	1626	2808	3982	4076	3159	1808	1560

Deoxyribonucleic acid analysis by flow cytometry of uterine leiomyosarcomas and smooth muscle tumors of uncertain malignant potential

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OBJECTIVES: The histologic distinction of uterine benign leiomyomas from leiomyosarcomas is difficult. Ploidy analysis and measurement of the proliferative rate were examined to determine if they could distinguish malignant from benign tumors and if they were independent prognostic factors.
STUDY DESIGN: Paraffin-embedded blocks were recut and prepared for flow cytometry with the technique of Hedley. Mitotic counts and tumor grading were performed on an adjacent hematoxylin and eosin–stained section. Statistical analysis was carried out with χ^2 life-table, and Cox model analysis.
RESULTS: There were 33 patients with deoxyribonucleic acid histograms that were acceptable for analysis. Fourteen tumors were diploid and 19 were aneuploid. There were no significant differences in the clinical characteristics between the patients with diploid tumors and those with aneuploid tumors. Aneuploid tumors were more likely to have cellular atypia ($p = 0.085$). There was a strong correlation between the percentage of cells in the S phase and the mitotic count ($p = 0.0001$). Increasing mitotic count, increasing S phase, presence of extrauterine disease, and postmenopausal status were all adverse prognostic factors. However, when multivariant analysis with a Cox model was used, only S phase and presence of extrauterine disease were adverse factors. Diploid tumors have a better overall survival ($p = 0.0658$) but a similiar disease-free survival. In those patients who ultimately have relapses, diploid tumors have a significantly longer interval from relapse to death ($p = 0.0045$).
CONCLUSIONS: Neither ploidy analysis nor measurement of the proliferative rate will distinguish a benign from a malignant course in an individual patient; however, ploidy is predictive of survival from time of disease progression and proliferative rate is the strongest predictor of overall survival. The time-proven reliability of mitotic count in the diagnosis of smooth muscle tumors reflects its ability to predict proliferative rate. (Am J Obstet Gynecol 1992;166:1646-54.)

Key words: Flow cytometry, proliferative rate, ploidy, uterine leiomyosarcomas

The bizarre appearance and marked atypia of many benign uterine smooth muscle tumors have made it difficult to establish absolute histologic criteria for the diagnosis of uterine leiomyosarcomas. With currently accepted definitions, tumors that are classified as malignant have a poor prognosis, with surival of 20% to 50% in many series.[1-5] To date the most reliable criterion for the diagnosis of leiomyosarcoma has been the mitotic count in the most active portion of the tumor.[1, 3, 4, 6, 7] Currently, there is no proved efficacious adjuvant therapy.

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6/6/37154

Deoxyribonucleic acid (DNA) analysis by flow cytometry has promise in establishing the diagnosis of benign versus malignant tumors and predicting the clinical course with the tumors. Identification of diploid tumors or tumors with a low proliferative rate (S phase) is of prognostic significance in other tumors, notably ovary and breast.[8-12] Uterine leiomyosarcomas with a diploid histogram have been shown to have a better prognosis than tumors with an aneuploid pattern.[13] The usefulness of flow cytometry in ovarian tumors of low malignant potential suggests a similar use in uterine smooth muscle tumors; that was the genesis for this study.

Material and methods

Archival material was obtained from four Seattle area hospitals that are members of the Puget Sound Oncology Consortium (Swedish Hospital Medical Center, University of Washington Medical Center, Virginia Mason Medical Center, and Overlake Medical Center) and from the University of Virginia Medical Center. Par-

affin-embedded blocks were recut in areas corresponding to the most aggressive-appearing portion of tumors previously identified as leiomyosarcomas or smooth muscle tumors of uncertain malignant potential. Formalin-fixed, paraffin-embedded sections of tissue were prepared for flow cytometric analysis with modifications of a previously published method.[14] Briefly, 50 μm sections were made from a prepresentative paraffin block of tumor tissue. Sections were deparaffinized, hydrated, treated with pepsin, pepstatin A, Triton X-100, and ribonuclease. Cells were then stained with propidium iodide as the DNA fluorochrome. DNA-stained nuclei were prepared and run on an Epics C flow cytometer (Coulter Electronics, Hialeah, Fla.). A total of 25,000 nuclear events were acquired on a single-parameter, 256-channel integrated fluorescence histogram.

A cell-cycle analysis software program (Multicycle, Phoenix Flow Systems, Inc., San Diego) was used to determine cell-cycle distributions of the presynthetic growth phase (G_0G_1), synthetic phase (S), and postsynthetic and mitotic growth phases (G_2M). DNA content was defined as aneuploid if two discrete G_0G_1 peaks were observed. In addition, the aneuploid G_0G_1 peak had to contain at least 10% of the 25,000 sample events and have a corresponding identifiable G_2M peak. Visual inspection of histograms was performed. Those with excess debris or insufficient resolution to distinguish two peaks were considered uninterpretable for S-phase and ploidy determination. In this study the coefficient of variation of the G_0G_1 peak of single-peak tumors was required to be <6 for the specimen to be considered interpretable for ploidy status.[8]

All tissue was reviewed by the study pathologist (D.R.H.) without knowledge of the clinical outcome. Malignancy grading was performed with a three-grade scale based on the classic grading system proposed by Broders and Hargrave.[15] Grading was determined primarily on cytomorphologic features (cell and nuclear pleomorphism) and degree of tissue differentiation.[16] Mitotic counts were performed after the method of O'Connor and Norris.[17] To determine mitotic activity, sections were scanned under low-power magnification to identify the most mitotically active areas. These areas were counted under high-power magnification (×400, area of field 0.159 mm²), and the highest number of mitoses in 10 consecutive random fields were recorded.

The classification system of Hendrickson and Kempson was used.[7] All tumors with >10 mitotic figures per 10 high-powered fields were considered leiomyosarcomas. Tumors with five to 10 mitotic figures per 10 high-powered fields were considered sarcomas if they had grade 3 of 3 cellular atypia and pleomorphism; otherwise, they were considered tumors of uncertain malignant potential. Hendrickson and Kempson also classify tumors with two to four mitotic figures per 10 high-powered fields with marked cellular atypia and pleomorphism as tumors of uncertain malignant potential. We did not have any patients with less than five mitotic figures per 10 high-powered fields for analysis.

Table I. Clinical characteristics in 33 women with leiomyosarcoma or smooth muscle tumors of uncertain malignancy potential

	Entire group	*Diploid*	*Aneuploid*
No. of patients	33	44	19
Median age (yr)	50	49	51
Race (% white)	88	86	89
Premenopausal (%)	48	57	42
Median parity	2	2	2
Extrauterine disease (stage III or IV) (%)	33	29	37

Table II. Correlation of histology and DNA analysis

	Entire group	*Diploid tumors*	*Aneuploid tumors*	*Significance*
Mean mitotic count	22.4	15.7	27.4	$p = 0.79$
Atypia (%)				
Grade 1	42.5	57.0	32.0	
Grade 2	42.5	43.0	42.0	
Grade 3	15.0	0.0	26.0	$p = 0.085$
Mean S phase (%)	12.4	9.2	14.8	$p = 0.47$

The Student t test and χ^2 were used to compare clinical characteristics and histologic features of diploid and aneuploid tumors. Proliferative rate and mitotic count were plotted and correlated with the Spearman rank coefficient. Kaplan-Meier life-table analysis was used, and differences in survival were compared with the log-rank method.[18, 19] Cox model regression was used to simultaneously compare multiple variables with survival as the dependent end point.[20]

Results

We identified 33 patients originally diagnosed as having leiomyosarcoma who had acceptable DNA histograms with a coefficient of variation for G_0G_1 of <6. Nineteen of these tumors were aneuploid and 14 were diploid. The clinical characteristics of these patients were presented in Table I. There were no significant differences in age, racial breakdown, menopausal status, parity, or presence of extrauterine disease between aneuploid and diploid tumors.

Table II presents the correlation between histologic data and DNA analysis. Aneuploid tumors were associated with a high degree of cellular atypia ($p = 0.085$). There was no correlation between ploidy status

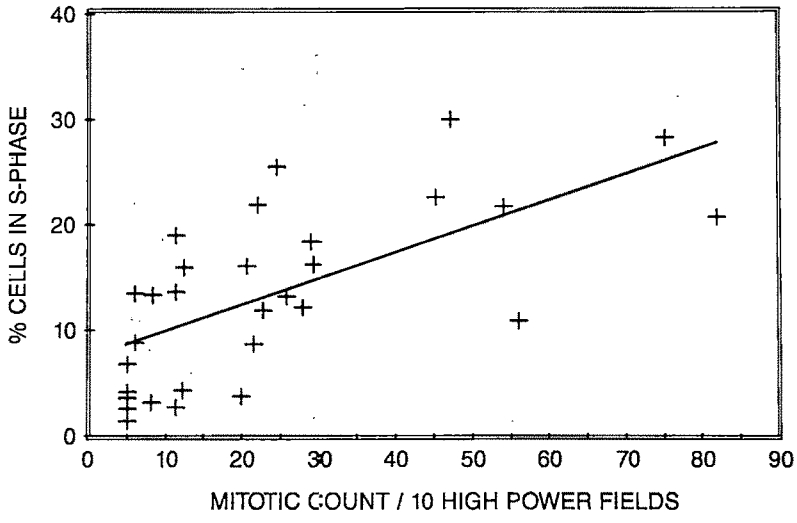


Fig. 1. Plot of S phase versus mitotic count and of linear regression of S phase predicted by mitotic count.

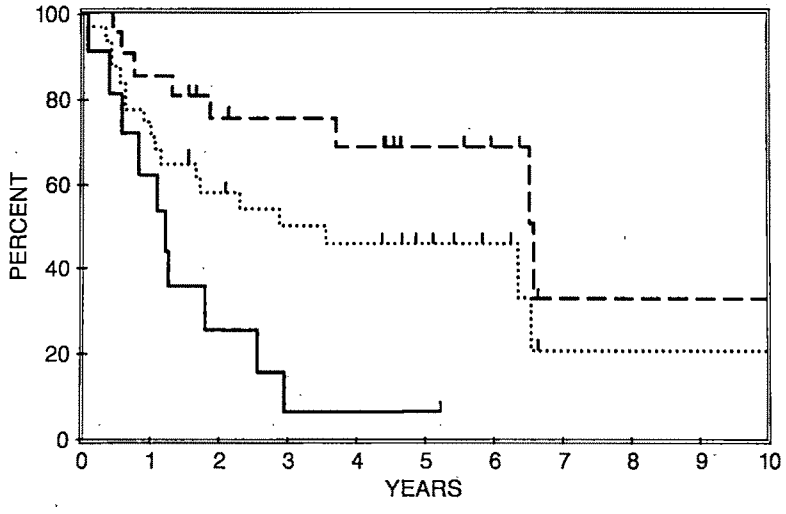


Fig. 2. Life-table survival probability for entire group and by presence or absence of extrauterine disease. *Dotted line*, All patients; *dashed line*, no extrauterine disease; *solid line*, extrauterine disease.

and mitotic count ($p = 0.79$) or between ploidy status and the percentage of cells in S phase ($p = 0.47$).

There was, however, a strong correlation between the percentage of cells in S phase and the mitotic count (Fig. 1). When S phase and mitotic count were plotted with linear regression, the mitotic count predicted 47% of the variation in S-phase measurements. The null hypothesis that there is no linear relationship is rejected at the 0.05 level ($p = 0.0001$).

Fig. 2 plots the overall survival and survival by the presence or absence of extrauterine disease. The median survival for the entire group was 43 months, with a projected 5-year survival of 49%. The presence of extrauterine disease at the time of clinical presentation was a strong clinical predictor of survival. The median survival of patients with extrauterine disease was 24 months and in patients without extrauterine disease was 79 months ($p = 0.0002$).

Fig. 3 plots survival by menopausal status. The survival advantage in premenopausal women approach statistical significance ($p = 0.0837$).

Fig. 4 presents the survival based on mitotic count. When patients are divided into three groups, there was a suggestion of a better survival with lower mitotic counts; this trend approached statistical significance when all patients with mitotic counts >10 were lumped together ($p = 0.0696$).

Fig. 5 is a plot of survival by the percentage of cells in S phase. There was a significant survival advantage for those patients with an S phase <10% ($p = 0.0221$).

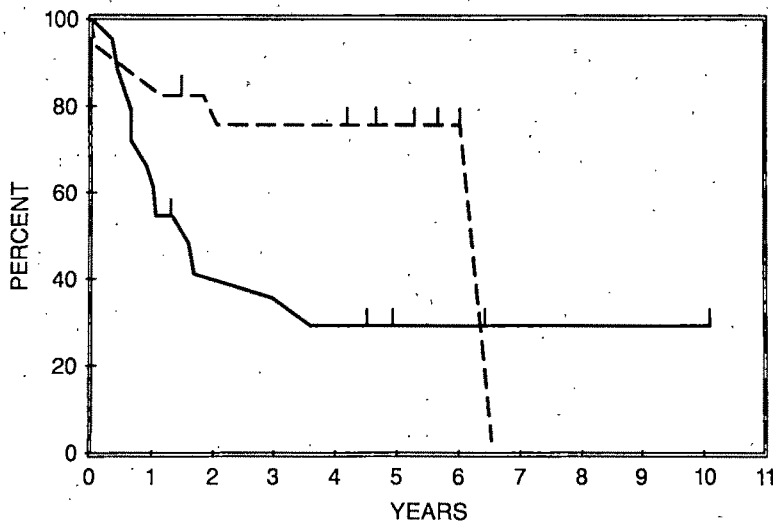


Fig. 3. Life-table survival probability by menopausal status. *Dashed line*, Premenopausal; *solid line*, postmenopausal.

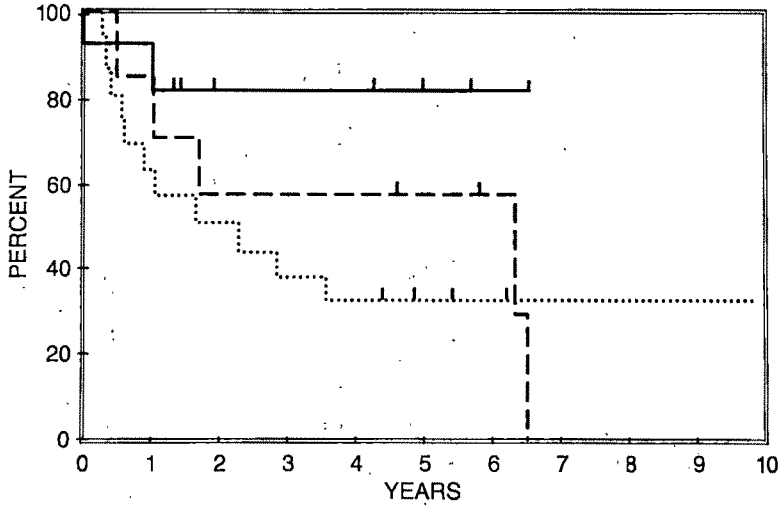


Fig. 4. Life-table survival probability by mitotic count. *Solid line*, 5 to 10; *dashed line*, 11 to 20; *dotted line*, >20.

Survival was also calculated with a break point of 12%, but in spite of moving three patients into the better prognosis group, there was almost no change in the plots ($p = 0.0271$). Because of the strong correlation between S phase and mitotic count, the two were compared in a Cox model, with survival as the dependent variable. S phase was shown to be the better independent predictor of survival (χ^2 7.95, $p = 0.0048$ vs χ^2 0.79, $p = 0.3740$).

S phase and presence or absence of extrauterine disease were compared in a separate Cox model. Both variables appeared to be independent predictors of survival (χ^2 7.21, $p = 0.0076$ for S phase; χ^2 6.73, $p = 0.0095$ for extrauterine disease). As a single variable, cellular atypia was a significant predictor of survival (χ^2 5.68, $p = 0.0200$); however, when examined in the Cox model with S phase and presence or absence of extrauterine disease, atypia was not an independent predictor of outcome (χ^2 0.02, $p = 0.8830$).

When postmenopausal status was tested in the Cox model with S phase and presence of extrauterine disease, menopausal status was not an adverse prognostic factor (χ^2 0.03, $p = 0.8619$), whereas presence of extrauterine disease (χ^2 5.25, $p = 0.0219$) and S phase (χ^2 6.99, $p = 0.0082$) remained independent prognostic factors.

Fig. 6 is a plot of survival based on ploidy analysis. There is a better survival in diploid tumors, with a median survival of 78.5 months versus 35.1 months with aneuploid tumors ($p = 0.0658$). Diploid tumors

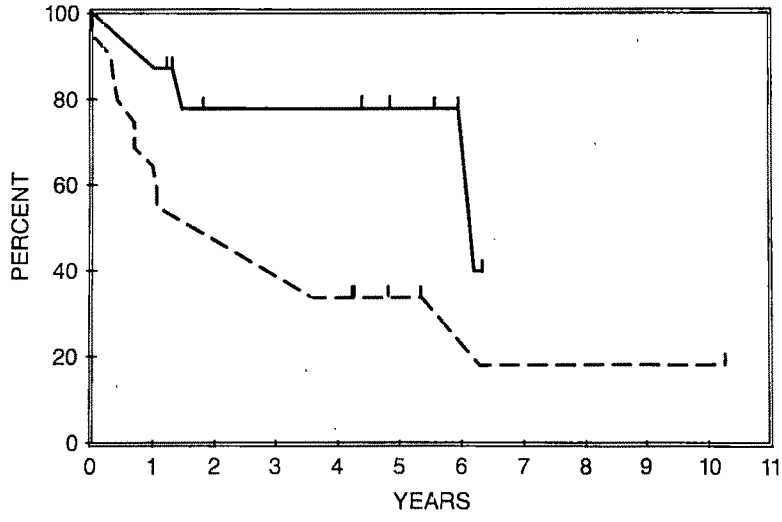


Fig. 5. Life-table survival probability by percentage of cells in S phase. *Solid line*, <10%; *dashed line*, ≥10%.

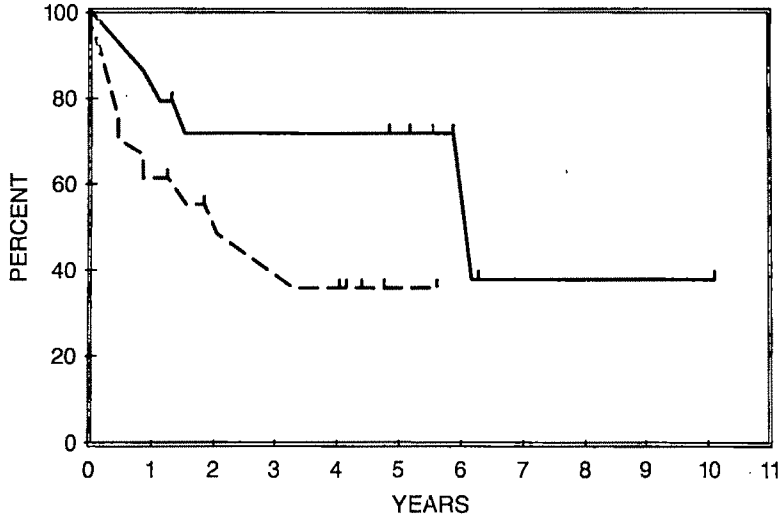


Fig. 6. Life-table survival probability by ploidy status. *Solid line*, Diploid; *dashed line*, aneuploid.

had a 70% 5-year survival and aneuploid tumors a 33% 5-year survival; however, when disease-free survival was plotted, there was no significant difference between diploid and aneuploid tumors (Fig. 7). This apparent discrepancy for survival between diploid and aneuploid tumors is explained by survival differences after recurrence, as plotted in Fig. 8. After development of a recurrence, patients with a diploid tumor have a superior survival ($p = 0.0045$).

There were 10 patients with a mitotic count of five to 10 per 10 high-power fields (Table III). None had grade 3 atypia and all were classified as tumors of uncertain malignant potential. Three of the 10 tumors were aneuploid; all three patients are alive and disease-free. Among the seven patients with diploid tumors, there have been four recurrences and one death. There is no clear-cut relationship between S phase and recurrence in these patients. When these patients have recurrence, they tend to have a more protracted course as compared with patients having leiomyosarcoma.

In an attempt to find an S-phase cutoff below which there were no recurrences or deaths, we looked separately at the 11 patients who had an S-phase total of <5%. In addition to patients 3, 7, and 8 listed in Table III, two other patients with an S phase of <5% have had recurrence. One had an S phase of 2.4% and a mitotic count of 11 per 10 high-power fields. She suffered a recurrence at 13 months and died at 36 months. The other patient had an S phase of 4.8% and a mitotic count of 12 per 10 high-power fields. A recurrence

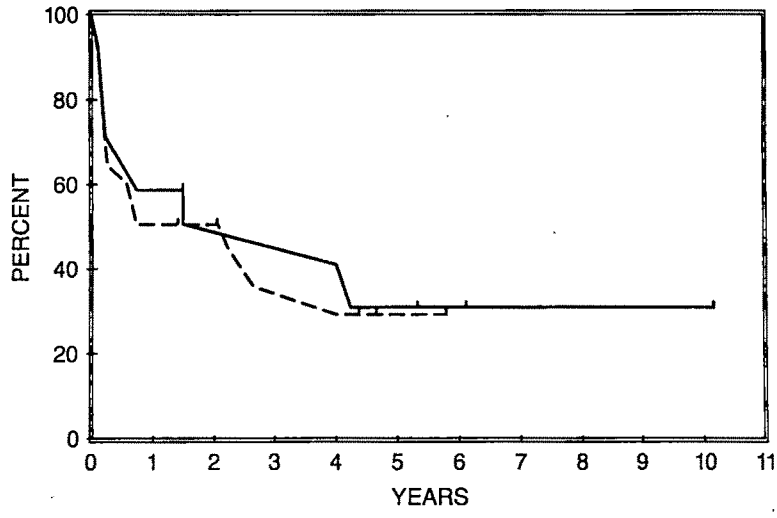


Fig. 7. Disease-free survival probability by ploidy status. *Solid line*, Diploid; *dashed line*, aneuploid.

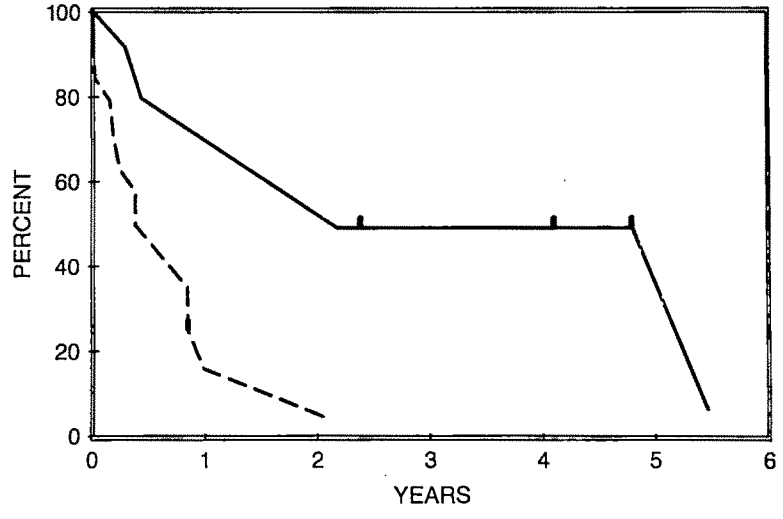


Fig. 8. Life-table survival probability from time of recurrence by ploidy status. *Solid line*, Diploid; *dashed line*, aneuploid.

developed at 53 months and she died at 77 months. Thus patients with two of the lowest three S-phase values have had recurrence. Among 11 patients who had an S phase >15%, 10 have had a recurrence.

Comment

Smooth muscle tumors of the uterus have presented problems both to the clinician and to the pathologist. Their clinical presentation is usually that of an enlarging mass, and unless there is presence of extrauterine disease, it is impossible to distinguish a sarcoma from a rapidly enlarging leiomyoma. Even the presence of extrauterine disease does not establish the diagnosis of malignancy, as there are well-documented cases of benign metastasizing leiomyomas, intravenous leiomyomatosis, and leiomyomatosis peritonealis disseminata. Clearly, there is a spectrum of disease from benign to malignant. Mitotic count has been the most consistent predictor, either alone or used in combination with atypia.[1,3,6] The criteria of Hendrickson and Kempson[7] acknowledge the uncertainty of the diagnosis and allow for a group of tumors of uncertain malignant potential. The patient's age and menopausal status also appear to have a bearing on the prognosis. Patients with postmenopausal status have generally had a poorer prognosis.[2,4,6] In our patients menopausal status was not an independent risk factor.

DNA analysis has proved useful in some but by no means all of the tumors in which it has been examined. It has become a recognized independent risk factor in

Table III. Histology and flow cytometry on 10 patients with tumors of uncertain malignant potential

Patient No.	*Age (yr)*	*Mitotic count/10 high-power fields*	*Atypia grade 1 to 3*	*Cells in S-phase (%)*	*Ploidy*	*Outcome*
1	76	5	2	3.6	Aneuploid	No evidence of disease 23 mo
2	37	5	1	4.8	Diploid	No evidence of disease 24 mo
3	51	5	1	4.8	Diploid	Recurrence 19 mo Alive with disease 74 mo
4	37	10	1	3.9	Aneuploid	No evidence of disease 61 mo
5	44	8	1	13.8	Aneuploid	No evidence of disease 53 mo
6	33	6	1	8.9	Diploid	Recurrence 3 mo Dead of disease 14 mo
7	68	8	1	3.0	Diploid	Recurrence 50 mo Alive with disease 77 mo
8	48	5	2	1.1	Diploid	Recurrence 2 mo Alive with disease 33 mo
9	39	5	1	4.1	Diploid	No evidence of disease 25 mo
10	47	5	1	6.7	Diploid	No evidence of disease 24 mo

node-negative breast cancer.[8] For uterine smooth muscle tumors Tsushima et al.[13] found a strong correlation between tumor grade and an abnormal DNA pattern. Survival was better in patients with diploid tumors, but they also found that 7% of patients classified as having benign leiomyoma had an aneuploid histogram and they cautioned that ploidy should not be used as an absolute criteria for the diagnosis of sarcoma. They did not present data on the implications of proliferative rate.

Our data underscore Tsushima's findings. Ploidy does seem to be an indicator of the aggressivenesss of the tumor. The finding of diploidy does not appear to have any ability to predict the benign versus malignant course of the disease, but once a patient had a relapse, diploid tumors tended to survive longer.

Proliferative rate (S-phase percentage) and mitotic count are strongly linked. This finding is not surprising, because both are indicators of the division rate of the individual cells. In the Cox model, S phase appeared to be the independent variable. An S phase >15% was associated with recurrence in 10 of 11 patients. Unfortunately, there was no lower level below which an S phase could be predictive of a benign course, and S-phase analysis made no contribution beyond knowledge of the mitotic count in prediction of the outcome of any individual patient. Although measurement of the S phase is an elegant test at the molecular level, mitotic count remains the gold standard for the diagnosis of leiomyosarcomas of the uterus. The effectiveness of mitotic count in classification of smooth muscle tumors probably reflects its ability to predict the proliferative rate. None of these patients had a response to either radiation therapy or chemotherapy and the only treatment modality that appeared to have any impact on their survival, once a recurrence was established, was surgical excision.

We thank Ruth Rice-Empniger, Page Kuhn, Daniel Hirschstein, and Joyce Shuck of the Laboratory of Pathology for technical expertise and efforts in performing the flow cytometry, and Tove Thompson and Janet O'Sullivan of the Puget Sound Oncology Consortium for data management and statistical analysis.

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Discussion

DR. PHILIP J. DISAIA, Irvine, California. I understand that the purpose of the investigation was to study the role of flow cytometry in predicting the behavior of leiomyosarcomas of the uterus and thereby assisting the clinician in prescribing therapy. This is a laudable goal, because these lesions behave in a very precarious manner in some patients and in an indolent manner in others.

The authors cite a previous study by Tsushima et al.[1] published in 1988 addressing the same subject; however, they classified the lesions into diploid versus polyploid versus aneuploid. Indeed, they found that 59% of the leiomyosarcomas were polyploid. Peters et al. chose not to use this classification but to divide their patients into diploid versus aneuploid. One wonders whether their results would have been different had they considered looking at the polyploid group separately. Also, the authors defined the criteria for aneuploidy as follows: "DNA content was defined as aneuploid if two discreet G_0G_1 peaks were observed." I would ask the authors why they felt that two distinct G_0G_1 peaks were necessary for the classification of aneuploidy. They quoted a study by Alvegard et al.,[2] which also included so-called G_0G_1 skews and G_0G_1 broadening as aneuploids. I would ask the authors whether using their criteria resulted in excluding samples and whether broadening the criteria for inclusion might have affected the results.

The authors found no significant differences in premenopausal status between the aneuploid and the diploid tumors. With 42% containing diploids and 58% containing aneuploids, I am curious whether this would hold true with a larger sample size. The same can be said for their finding that there was no correlation between ploidy status and mitotic count ($p = 0.79$) or between ploidy status and percentage of cells in S phase ($p = 0.47$). Interpretation of p values with such small sample sizes is difficult at best.

The conclusions were consistent with those of the largest study in the literature, that of Tsushima et al.[1] We are left with a traditional way of counting mitotic figures. However, O'Connor and Norris[3] have suggested adding the observation of atypia to this calculation of mitotic figures (Table I). They studied 73 smooth muscle tumors of the uterus with five to nine mitotic figures per 10 high-power fields and concluded that mitotically active smooth muscle tumors of the uterus having five to nine mitoses per 10 high-power fields and no cellular atypia have a metastatic rate too low to be regarded as sarcomas. Follow-up of their patients from 23 months to 15 years (median 7 years) gives great importance to their conclusions. Patients with atypia or >10 mitotic figures per 10 high-power fields had the potential for metastic lesions. Until we refine the techniques of flow cytometry or have comparable technology at hand, I believe this classification should prevail.

The major criticism of this article has to do with the small sample size. This good work should be considered as a basis for a larger study, possibly by a national cooperative group, to properly test the basis of their conclusions.

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Table I. Metastatic potential of smooth muscle tumors of uterus

Maximum mitotic figures/10 high-power fields	*Atypia*	*Diagnosis*	*Metastatic potential*
1-4	Any degree	Leiomyoma	Very low
5-9	None	Leiomyoma with high mitotic activity	Very low
5-9	Grade 1*	Smooth muscle tumor of uncertain malignant potential	Low
5-9	Grade 2 or 3*	Leiomysarcoma	Moderate
≥10	Grade 1*	Leiomysarcoma	High
≥10	Grade 2 or 3*	Leiomysarcoma	Very high

Modified from O'Connor DM, Norris HJ. Mitotically active leiomyomas of the uterus. Hum Pathol 1990;21:223-7.
*Grade based on a scale of three.

usefulness of nuclear DNA patterns studied by flow cytometry. Mayo Clin Proc 1988;63:248-55.
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Dr. Peters (Closing). A technical issue to be addressed in a flow cytometry study is how wide a coefficient of variation to accept. The answer determines whether you get narrow, sharp histograms, like the one I showed, or ones that are quite wide. One of my coauthors, Dr. Donald Howard, a pathologist, insisted on a coefficient of variation of 6, which is much smaller than many other authors have used. Doing that eliminates many wide G_0G_1 peaks that would have been classified as near aneuploid. The temptation exists to liberalize the criteria and include as many subjects as possible. Enlarging the group this way may help in statistical analysis, but, from the point of view of the molecular biologist, the data are tainted. In the case of our data we have also analyzed them with coefficients of variation of 8, 10, and 12, but the number that would be reclassified as aneuploid was fairly small.

We were surprised to find no polyploid lesions in our series; we had expected to see about 10%. Tsushima et al.[1] had a very high proportion of patients with tetraploid tumors. Although we expected to see a few in our series, we certainly did not expect to see 50%.

Menopausal status has been a significant prognostic variable in many previous series, and in our series, when we looked at it as a single variable, it did appear to be a prognostic variable. However, in the Cox model, which allows us to sort out the independent variables by multivariate regression, there was no suggestion that menopausal status was important.

Dr. DiSaia, I agree that atypia is an important diagnostic criterion in the classification system of Hendrickson and Kempson.[2] I also totally agree that some form of effective chemotherapy for these patients is needed because the end results have been so poor. We have previously presented our results of adjuvant chemotherapy of mixed mesodermal tumors with a combination of cisplatin and doxorubicin and it was quite effective.[3] Our experience with this regimen in leiomyosarcomas, however, has been dismal. We have not seen responses in patients with metastatic disease, nor do we appear to have had a prolongation of disease-free survival. We need nationwide randomized studies of other combinations.

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Serious as well as minor side effects have been reported following the use of oral contraceptives. These include thrombo-embolic disease.

Please see adjacent page for brief summary of prescribing information.

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TABLETS

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DESCRIPTION

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TRI-NORINYL 28-DAY Tablets provide a continuous oral contraceptive regimen of 7 blue tablets, 9 yellow-green tablets, 5 more blue tablets, and then 7 orange tablets. Each blue tablet contains norethindrone 0.5 mg and ethinyl estradiol 0.035 mg, each yellow-green tablet contains norethindrone 1 mg and ethinyl estradiol 0.035 mg, and each orange tablet contains inert ingredients.

INDICATIONS AND USAGE

Oral contraceptives are indicated for the prevention of pregnancy in women who elect to use these products as a method of contraception.

CONTRAINDICATIONS

Oral contraceptives should not be used in women who have the following conditions: Thrombophlebitis or thromboembolic disorders • A past history of deep vein thrombophlebitis or thromboembolic disorders • Cerebral vascular or coronary artery disease • Known or suspected carcinoma of the breast • Carcinoma of the endometrium, and known or suspected estrogen-dependent neoplasia • Undiagnosed abnormal genital bleeding • Cholestatic jaundice of pregnancy or jaundice with prior pill use • Hepatic adenomas, carcinomas or benign liver tumors • Known or suspected pregnancy

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risks of several serious conditions including myocardial infarction, thromboembolism, stroke, hepatic neoplasia and gallbladder disease, although the risk of serious morbidity and mortality increases significantly in the presence of other underlying risk factors such as hypertension, hyperlipidemias, hypercholesterolemia, obesity and diabetes.

Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

The information contained in this package insert is principally based on studies carried out in patients who used oral contraceptives with formulations containing 0.05 mg or higher of estrogen. The effects of long-term use with lower dose formulations of both estrogens and progestogens remain to be determined.

Throughout this labeling, epidemiological studies reported are of two types: retrospective or case control studies and prospective or cohort studies. Case control studies provide a measure of the relative risk of a disease. Relative risk, the *ratio* of the incidence of a disease among oral contraceptive users to that among non-users, cannot be assessed directly from case control studies, but the odds ratio obtained is a measure of relative risk. The relative risk does not provide information on the actual clinical occurrence of a disease. Cohort studies provide not only a measure of the relative risk but a measure of attributable risk, which is the *difference* in the incidence of disease between oral contraceptive users and non-users. The attributable risk does provide information about the actual occurrence of a disease in the population.

1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS

a. Myocardial infarction

An increased risk of myocardial infarction has been attributed to oral contraceptive use. This risk is primarily in smokers or women with other underlying risk factors for coronary artery disease such as hypertension, hypercholesterolemia, morbid obesity and diabetes. The relative risk of heart attack for current oral contraceptive users has been estimated to be 2 to 6. The risk is very low under the age of 30. However, there is the possibility of a risk of cardiovascular disease even in very young women who take oral contraceptives.

Smoking in combination with oral contraceptive use has been shown to contribute substantially to the incidence of myocardial infarctions in women 35 or older, with smoking accounting for the majority of excess cases.

Mortality rates associated with circulatory disease have been shown to increase substantially in smokers over the age of 35 and non-smokers over the age of 40 among women who use oral contraceptives.

Oral contraceptives may compound the effects of well-known risk factors for coronary artery disease, such as hypertension, diabetes, hyperlipidemias, hypercholesterolemia, age and obesity. In particular, some progestogens are known to decrease HDL cholesterol and impair oral glucose tolerance, while estrogens may create a state of hyperinsulinism. Oral contraceptives have been shown to increase blood pressure among users (see **WARNINGS**, section 9). Similar effects on risk factors have been associated with an increased risk of heart disease. Oral contraceptives must be used with caution in women with cardiovascular disease risk factors.

b. Thromboembolism

An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Case control studies have found the relative risk of users compared to non-users to be 3 for the first episode of superficial venous thrombosis, 4 to 11 for deep vein thrombosis or pulmonary embolism, and 1.5 to 6 for women with predisposing conditions for venous thromboembolic disease. One cohort study has shown the relative risk to be somewhat lower, about 3 for new cases (subjects with no past history of venous thrombosis or varicose veins) and about 4.5 for new cases requiring hospitalization. The risk of thromboembolic disease due to oral contraceptives is not related to length of use and disappears after pill use is stopped.

A 2- to 6-fold increase in relative risk of post-operative thromboembolic complications has been reported with the use of oral contraceptives. If feasible, oral contraceptives should be discontinued at least 4 weeks prior to and for 2 weeks after elective surgery and during and following prolonged immobilization. Since the immediate postpartum period also is associated with an increased risk of thromboembolism, oral contraceptives should be started no earlier than 4 to 6 weeks after delivery in women who elect not to breast feed.

c. Cerebrovascular diseases

An increase in both the relative and attributable risks of cerebrovascular events (thrombotic and hemorrhagic strokes) has been shown in users of oral contraceptives. In general, the risk is greatest among older (>35 years), hypertensive women who also smoke. Hypertension was found to be a risk factor for both users and non-users for both types of strokes while smoking interacted to increase the risk for hemorrhagic strokes.

In a large study, the relative risk of thrombotic strokes has been shown to range from 3 for normotensive users to 14 for users with severe hypertension. The relative risk of hemorrhagic stroke is reported to be 1.2 for non-smokers who used oral contraceptives, 2.6 for smokers who did not use oral contraceptives, 7.6 for smokers who used oral contraceptives, 1.8 for normotensive users and 25.7 for users with severe hypertension. The attributable risk also is greater in women 35 or older and among smokers.

d. Dose-related risk of vascular disease from oral contraceptives

A positive association has been observed between the amount of estrogen and progestogen in oral contraceptives and the risk of vascular disease. A decline in serum high density lipoproteins (HDL) has been reported with some progestational agents. A decline in serum high density lipoproteins has been associated with an increased incidence of ischemic heart disease. Because estrogens increase HDL cholesterol, the net effect of an oral contraceptive depends on a balance achieved between doses of estrogen and progestogen and the nature and absolute amount of progestogens used in the contraceptives. The amount of both hormones should be considered in the choice of an oral contraceptive.

Minimizing exposure to estrogen and progestogen is in keeping with good principles of therapeutics. For any particular estrogen/progestogen combination, the dosage regimen prescribed should be one which contains the least amount of estrogen and progestogen that is compatible with a low failure rate and the needs of the individual patient. New acceptors of oral contraceptive agents should be started on preparations containing the lowest estrogen content that produces satisfactory results for the individual.

e. Persistence of risk of vascular disease

There are three studies which have shown persistence of risk of vascular disease for ever-users of oral contraceptives. In a study in the United States, the risk of developing myocardial infarction after discontinuing oral contraceptives persists for at least 9 years for women 40–49 years who had used oral contraceptives for 5 or more years, but this increased risk was not demonstrated in other age groups. In another study in Great Britain, the risk of developing cerebrovascular disease persisted for at least 6 years after discontinuation of oral contraceptives, although excess risk was very small. Subarachnoid hemorrhage also has a significantly increased relative risk after termination of use of oral contraceptives. However, these studies were performed with oral contraceptive formulations containing 0.05 mg or higher of estrogen.

2. ESTIMATES OF MORTALITY FROM CONTRACEPTIVE USE

One study gathered data from a variety of sources which have estimated the mortality rates associated with different methods of contraception at different ages. These estimates include the combined risk of death associated with contraceptive methods plus the risk attributable to pregnancy in the event of method failure. Each method of contraception has its specific benefits and risks. The study concluded that with the exception of oral contraceptive users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of birth control is low and below that associated with childbirth. The observation of a possible increase in risk of mortality with age for oral contraceptive users is based on data gathered in the 1970s—but not reported in the U.S. until 1983. However, current clinical practice involves the use of lower estrogen dose formulations combined with careful restriction of oral contraceptive use to women who do not have the various risk factors listed in this labeling.

Because of these changes in practice and, also, because of some limited new data which suggest that the risk of cardiovascular disease with the use of oral contraceptives may now be less than previously observed, the Fertility and Maternal Health Drugs Advisory Committee was asked to review the topic in 1989. The Committee concluded that although cardiovascular disease risks may be increased with oral contraceptive use after age 40 in healthy non-smoking women (even with the newer low-dose formulations), there are greater potential health risks associated with pregnancy in older women and with the alternative surgical and medical procedures which may be necessary if such women do not have access to effective and acceptable means of contraception.

Therefore, the Committee recommended that the benefits of oral contraceptive use by healthy non-smoking women over 40 may outweigh the possible risks. Of course, older women, as all women who take oral contraceptives, should take the lowest possible dose formulation that is effective.

3. CARCINOMA OF THE BREAST AND REPRODUCTIVE ORGANS

Numerous epidemiological studies have been performed on the incidence of breast, endometrial, ovarian and cervical cancer in women using oral contraceptives. The evidence in the literature suggests that use of oral contraceptives is not associated with an increase in the risk of developing breast cancer, regardless of the age and parity of first use or with most of the marketed brands and doses. The Cancer and Steroid Hormone study also showed no latent effect on the risk of breast cancer for at least a decade following long-term use. A few studies have shown a slightly increased relative risk of developing breast cancer, although the methodology of these studies, which included differences in examination of users and non-users and differences in age at start of use, has been questioned. Some studies have reported an increased relative risk of developing breast cancer, particularly at a younger age. This increased relative risk appears to be related to duration of use.

Some studies suggest that oral contraceptive use has been associated with an increase in the risk of cervical intraepithelial neoplasia in some populations of women. However, there continues to be controversy about the extent to which such findings may be due to differences in sexual behavior and other factors.

In spite of many studies of the relationship between oral contraceptive use and breast or cervical cancers, a cause and effect relationship has not been established.

4. HEPATIC NEOPLASIA

Benign hepatic adenomas are associated with oral contraceptive use although the incidence of benign tumors is rare in the United States. Indirect calculations have estimated the attributable risk to be in the range of 3.3 cases per 100,000 for users, a risk that increases after 4 or more years of use. Rupture of rare, benign, hepatic adenomas may cause death through intra-abdominal hemorrhage.

Studies in the United States and Britain have shown an increased risk of developing hepatocellular carcinoma in long-term (>8 years) oral contraceptive users. However, these cancers are extremely rare in the United States and the attributable risk (the excess incidence) of liver cancers in oral contraceptive users is less than 1 per 1,000,000 users.

5. OCULAR LESIONS

There have been clinical case reports of retinal thrombosis associated with the use of oral contraceptives. Oral contraceptives should be discontinued if there is unexplained partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions. Appropriate diagnostic and therapeutic measures should be undertaken immediately.

6. ORAL CONTRACEPTIVE USE BEFORE OR DURING EARLY PREGNANCY

Extensive epidemiological studies have revealed no increased risk of birth defects in women who have used oral contraceptives prior to pregnancy. More recent studies do not suggest a teratogenic effect, particularly insofar as cardiac anomalies and limb reduction defects are concerned, when taken inadvertently during early pregnancy.

The administration of oral contraceptives to induce withdrawal bleeding should not be used as a test for pregnancy. Oral contraceptives should not be used during pregnancy to treat threatened or habitual abortion.

It is recommended that for any patient who has missed 2 consecutive periods, pregnancy should be ruled out before continuing oral contraceptive use. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period. Oral contraceptive use should be discontinued if pregnancy is confirmed.

7. GALLBLADDER DISEASE

Earlier studies have reported an increased lifetime relative risk of gallbladder surgery in users of oral contraceptives and estrogens. More recent studies, however, have shown that the relative risk of developing gallbladder disease among oral contraceptive users may be minimal. The recent findings of minimal risk may be related to the use of oral contraceptive formulations containing lower hormonal doses of estrogens and progestogens.

8. CARBOHYDRATE AND LIPID METABOLIC EFFECTS

Oral contraceptives have been shown to impair oral glucose tolerance. Oral contraceptives containing greater than 0.075 mg of estrogen cause glucose intolerance with impaired insulin secretion, while lower doses of estrogen may produce less glucose intolerance. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents. However, in the non-diabetic woman, oral contraceptives appear to have no effect on fasting blood glucose. Because of these demonstrated effects, prediabetic and diabetic women should be carefully observed while taking oral contraceptives.

Some women may develop persistent hypertriglyceridemia while on the pill. As discussed earlier (see **WARNINGS**, sections 1a. and 1d.), changes in serum triglycerides and lipoprotein levels have been reported in oral contraceptive users.

9. ELEVATED BLOOD PRESSURE

An increase in blood pressure has been reported in women taking oral contraceptives. The incidence of risk also was reported to increase with continued use and among older women. Data from the Royal College of General Practitioners and subsequent randomized trials have shown that the incidence of hypertension increases with increasing concentrations of progestogens.

Women with a history of hypertension or hypertension-related diseases or renal disease should be encouraged to use another method of contraception. If women elect to use oral contraceptives, they should be monitored closely and if significant elevation of blood pressure occurs oral contraceptives should be discontinued. For most women, elevated blood pressure will return to normal after stopping oral contraceptives and there is no difference in the occurrence of hypertension among ever- and never-users.

10. HEADACHE

The onset or exacerbation of migraine or development of headache with a new pattern which is recurrent, persistent or severe requires discontinuation of oral contraceptives and evaluation of the cause.

11. BLEEDING IRREGULARITIES

Breakthrough bleeding and spotting are sometimes encountered in patients on oral contraceptives, especially during the first 3 months of use. Non-hormonal causes should be considered and adequate diagnostic measures taken to rule out malignancy or pregnancy in the event of breakthrough bleeding, as in the case of any abnormal vaginal bleeding. If pathology has been excluded, time or a change to another formulation may solve the problem. In the event of amenorrhea, pregnancy should be ruled out.

Some women may encounter post-pill amenorrhea or oligomenorrhea, especially when such a condition was pre-existent.

PRECAUTIONS

1. PHYSICAL EXAMINATION AND FOLLOW-UP

A complete medical history and physical examination should be taken prior to the initiation or reinstitution of oral contraceptives and at least annually during use of oral contraceptives. These physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including cervical cytology and relevant laboratory tests. In case of undiagnosed, persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be conducted to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care.

2. LIPID DISORDERS

Women who are being treated for hyperlipidemias should be followed closely if they elect to use oral contraceptives. Some progestogens may elevate LDL levels and may render the control of hyperlipidemias more difficult.

3. LIVER FUNCTION

If jaundice develops in any woman receiving oral contraceptives the medication should be discontinued. Steroid hormones may be poorly metabolized in patients with impaired liver function.

4. FLUID RETENTION

Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention.

5. EMOTIONAL DISORDERS

Women with a history of depression should be carefully observed and the drug discontinued if depression recurs to a serious degree.

6. CONTACT LENSES

Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist.

7. DRUG INTERACTIONS

Reduced efficacy and increased incidence of breakthrough bleeding and menstrual irregularities have been associated with concomitant use of rifampin. A similar association though less marked, has been suggested with barbiturates, phenylbutazone, phenytoin sodium, and possibly with griseofulvin, ampicillin and tetracyclines.

8. INTERACTIONS WITH LABORATORY TESTS

Certain endocrine and liver function tests and blood components may be affected by oral contraceptives:

a. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.

b. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG. Free T4 concentration is unaltered.

c. Other binding proteins may be elevated in serum.

d. Sex steroid binding globulins are increased and result in elevated levels of total circulating sex steroids and corticoids; however, free or biologically active levels remain unchanged.

e. Triglycerides may be increased.

f. Glucose tolerance may be decreased.

g. Serum folate levels may be depressed by oral contraceptive therapy. This may be of clinical significance if a woman becomes pregnant shortly after discontinuing oral contraceptives.

9. CARCINOGENESIS

See **WARNINGS** section.

10. PREGNANCY

Pregnancy Category X. See **CONTRAINDICATIONS** and **WARNINGS** sections.

11. NURSING MOTHERS

Small amounts of oral contraceptive steroids have been identified in the milk of nursing mothers and a few adverse effects on the child have been reported, including jaundice and breast enlargement. In addition, oral contraceptives given in the postpartum period may interfere with lactation by decreasing the quantity and quality of breast milk. If possible, the nursing mother should be advised not to use oral contraceptives but to use other forms of contraception until she has completely weaned her child.

INFORMATION FOR THE PATIENT

See **PATIENT LABELING.**

ADVERSE REACTIONS

An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see **WARNINGS** section): Thrombophlebitis • Arterial thromboembolism • Pulmonary embolism • Myocardial infarction • Cerebral hemorrhage • Cerebral thrombosis • Hypertension • Gallbladder disease • Hepatic adenomas, carcinomas or benign liver tumors

There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: Mesenteric thrombosis • Retinal thrombosis

The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea • Vomiting • Gastrointestinal symptoms (such as abdominal cramps and bloating) • Breakthrough bleeding • Spotting • Change in menstrual flow • Amenorrhea • Temporary infertility after discontinuation of treatment • Edema • Melasma which may persist • Breast changes: tenderness, enlargement, secretion • Change in weight (increase or decrease) • Change in cervical erosion and secretion • Diminution in lactation when given immediately postpartum • Cholestatic jaundice • Migraine • Rash (allergic) • Mental depression • Reduced tolerance to carbohydrates • Vaginal candidiasis • Change in corneal curvature (steepening) • Intolerance to contact lenses

The following adverse reactions have been reported in users of oral contraceptives and the association has been neither confirmed nor refuted: Pre-menstrual syndrome • Cataracts • Changes in appetite • Cystitis-like syndrome • Headache • Nervousness • Dizziness • Hirsutism • Loss of scalp hair • Erythema multiforme • Erythema nodosum • Hemorrhagic eruption • Vaginitis • Porphyria • Impaired renal function • Hemolytic uremic syndrome • Budd-Chiari syndrome • Acne • Changes in libido • Colitis

OVERDOSAGE

Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

NON-CONTRACEPTIVE HEALTH BENEFITS

The following non-contraceptive health benefits related to the use of oral contraceptives are supported by epidemiological studies which largely utilized oral contraceptive formulations containing estrogen doses exceeding 0.035 mg of ethinyl estradiol or 0.05 mg of mestranol.

Effects on menses: • Increased menstrual cycle regularity • Decreased blood loss and decreased incidence of iron deficiency anemia • Decreased incidence of dysmenorrhea

Effects related to inhibition of ovulation: • Decreased incidence of functional ovarian cysts • Decreased incidence of ectopic pregnancies

Effects from long-term use: • Decreased incidence of fibroadenomas and fibrocystic disease of the breast • Decreased incidence of acute pelvic inflammatory disease • Decreased incidence of endometrial cancer • Decreased incidence of ovarian cancer

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The utility of immunocytochemistry in invasive adenocarcinoma of the cervix

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OBJECTIVES: The purpose of the study was to evaluate the utility of immunocytochemistry in the management of cervical adenocarcinoma. Patterns of reactivity to various immunocytochemical markers were correlated with clinically known risk factors for recurrence.

STUDY DESIGN: A retrospective evaluation of 55 patients with invasive cervical adenocarcinoma was conducted. Monoclonal antibodies were generated against various human intermediate filament proteins and tested on fixed, paraffin-embedded tissue by the avidin-biotin complex immunoperoxidase method. Dako V9 antivimentin antibodies and two anticytokeratin antibodies, 35βH11 and 34βE12, were used. Polyclonal antibodies to carcinoembryonic antigen and antibodies to epithelial membrane antigen were used.

RESULTS: Of 55 patients, 50 (91%) had positive epithelial membrane antigen antibodies in tumor cells. Tissue from patients with cervical adenocarcinoma demonstrated positivity for 35βH11 antibodies in 46 of 53 patients (87%) and positivity for 34βE12 antibodies in 15 of 54 patients (28%). Vimentin antibodies were negative in all except one patient. Carcinoembryonic antigen–positive antibodies were detected in 37 of 55 patients (67%). This study also demonstrated consistent negative expression of vimentin in invasive cervical adenocarcinoma. Carcinoembryonic antigen expression rates were 50% and 75% for patients with small tumors (≤2 cm) and large tumors (>2 cm), respectively ($p < 0.01$). Patients with carcinoembryonic antigen expression are at increased risk for recurrence and also sustain reduced survival potential.

CONCLUSIONS: Patients with cervical adenocarcinoma and carcinoembryonic antigen expression are at increased risk for recurrence. We speculate that immunocytochemistry may detect lymph node micrometastases otherwise unsuspected by conventional microscopy. (Am J Obstet Gynecol 1992;166:1655-62.)

Key words: Immunocytochemistry, cervical adenocarcinoma

The diagnosis of invasive adenocarcinoma of the cervix as a distinct clinical entity poses special problems for the epidemiologist, pathologist, surgeon, and radiotherapist. Several reports have observed an increasing incidence of adenocarcinoma of the cervix,[1-3] particularly in the last three decades. It is unknown whether this is an absolute or relative increased incidence. The effectiveness of conservative outpatient therapy for squamous cell cervical intraepithelial neoplasia may have resulted in increased relative incidence of cervical adenocarcinoma as compared with invasive squamous cell carcinoma. There is a disproportionately higher incidence of adenocarcinoma among patients with development of invasive cervical cancer after unsuccessful outpatient treatment for cervical intraepithelial neoplasia.[4] Moreover, cytologic screening for detection of invasive cervical adenocarcinoma has proved less than satisfactory.[5]

Classifications of adenocarcinoma of the cervix have been introduced in an attempt to assign risk factors related to the histopathologic diagnosis. Several observers have concluded that patients with the diagnosis of adenosquamous and poorly differentiated adenocarcinoma[6, 7] are probably at higher risk for recurrence and hence sustain lower survival rates. Histochemical studies, such as mucin stains, have failed to define a marker that can reliably distinguish between an endocervical and endometrial primary tumor.[8] Current interest in immunocytochemistry, however, has reawakened interest in this distinction, and it has been demonstrated that antibodies to vimentin, intermediate filament protein, provide an immunocytochemical tool that enables the pathologist to distinguish between adenocarcinoma of the uterine cervix and adenocarcinoma of the endometrium.[9] Mammalian cells contain three classes of cytoplasmic filamentous structures: mi-

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crofilaments, microtubules, and filaments of intermediate diameter. Intermediate filaments are divided into various subclasses on the basis of biochemical analysis and immunochemical reactivity.[10, 11] Cytokeratins are the intermediate filaments present in epithelial cells; mesenchymal cells contain the intermediate filament protein vimentin. Desmin, glial fibrillary acidic protein, and neurofilaments are other examples of intermediate filament subclasses. Intermediate filament proteins may be used as markers to identify specific cell types in benign or malignant tumors. Malignant cells, in general, contain the same intermediate filament class as in corresponding normal tissue.[12] Monoclonal or polyclonal antibodies generated against the various human intermediate filament proteins can be used in deparaffinized sections or formalin-fixed tissues.

The purpose of this report is to determine the utility of immunocytochemistry in detecting tissue cell markers in 55 patients with cervical adenocarcinoma and to correlate the clinical aspects of this disease with the various immunocytochemical findings. The following immunocytochemical proteins were used: vimentin, cytokeratin 8 (low-molecular-weight cytokeratin), high-molecular-weight cytokeratin, carcinoembryonic antigen, and epithelial membrane antigen.

Material and methods

The files of the Division of Gynecologic Oncology at the University of Washington Medical Center were reviewed. During the 10-year period from 1976 through 1985, 88 patients with the diagnosis of invasive cervical adenocarcinoma and its subtypes were identified. Patients with the diagnosis of adenocarcinoma in situ were not considered in this study. The cases included in the study had tissue blocks from radical hysterectomies, total abdominal hysterectomies, total pelvic exenterations, or cervical biopsies. Thirty-three of the 88 patients were excluded from the study because of lack of suitable histologic material. In 21 instances outside hospital surgical specimens were not available. In three instances no tumor remained after processing, and in nine cases the tissue available proved unsuitable for immunocytochemical study, because of either inconsistent immunostaining or poor antigenic preservation of the specimen. Fifty-five cases were thus included in the study.

All surgical specimens had been previously fixed in formalin and paraffin embedded in blocks. In 10% of the cases a portion of the tissues had also been fixed in methacarn.

Sections measuring 5 μm were deparaffinized in xylene and rehydrated in a series of graded alcohol incubations. Serial sections of each case were then incubated with a panel of monoclonal antibodies consisting of 35βH11, an anticytokeratin 8 antibody; 34βE12, an anticytokeratin monoclonal antibody[13]; V9, an antivimentin monoclonal antibody; E29, an antiepithelial membrane antigen monoclonal antibody; and a rabbit polyclonal antibody to carcinoembryonic antigen. All antibodies were obtained from, or are available from, Dako Corporation (Carpinteria, Calif.). In the case of 35βH11 and 34βE12 only, predigestion of the tissue sections before antibody incubation was required (20 minutes of 0.01% pronase in phosphate-buffered saline solution [Calbiochem, La Jolla, Calif.] and 10 minutes of 0.05% Type II trypsin [Sigma, St. Louis] in 0.05 mol/L Tris buffer, pH 7.6, respectively). All antibody localization was by a modification of the avidin-biotin immunoperoxidase technique, as described,[13] in which nickel chloride is added to the chromogen, 3,3′-diaminobenzidine, to yield a black reaction product.

The hospital records of the 55 patients involved in the study were reviewed for age, parity, method of contraception, stage, grade, histologic type, symptoms, vaginal cytologic results, presence or absence of gross lesion, survival, follow-up duration, recurrence, primary therapy, grade of tumor, lesion size, depth of penetration, nodal metastases, number of metastatic nodes, associated cervical intraepithelial neoplasia, and adjuvant therapy. To maintain consistency in estimating tumor size, the measurements were obtained from pathologic evaluation when possible. In patients receiving preoperative irradiation, the measurements were estimated from clinical data. The minimum follow-up duration was 60 months. A $p < 0.05$ was considered statistically significant.

Results

Clinical profile. The mean age of the patients in the study was 39.6 years and the median age was 37 years. Forty-one of the 55 patients were treated by radical hysterectomy and bilateral lymphadenectomy; these patients form a subgroup that yielded more precise information regarding depth of invasion and metastatic spread. These patients were also slightly younger, with a mean age of 36.1 years and a median age of 36 years. Parity ranged from nulliparity to para 8, with a mean of 2.5 and a median of 2.

The stage of the cervical cancer was determined according to the International Federation of Gynecology and Obstetrics staging system as revised in 1988. None of the cases were considered microinvasive. Forty-five were stage IB, five stage II, two stage III, and three patients had recurrent or persistent disease after initial therapy performed elsewhere.

Adenocarcinoma was the histologic diagnosis in 43 of the patients, adenosquamous in 10, mesonephric (wolffian duct) adenocarcinoma in one, and poorly differentiated papillary adenocarcinoma in one. Bleeding was the most common symptom and was experienced in 36 (65.45%) of cases. Eighteen (32.73%) denied any presenting symptom, and one patient complained of excessive vaginal discharge.

Table I. Immunocytochemical analysis of various antibodies

Antibody	Positive tumor staining		Positive internal control	
	No.	%	No	%
Anticytokeratin				
35βH11	46/53	87	48/53	91
34βE12	15/54	28	45/52	85
Antivimentin	01/54	1.8	42/55	76
Anti-carcinoembryonic-antigen	37/55	67	49/53	92
Anti-epithelial-membrane-antigen	50/55	91	55/55	100

Table II. Age versus immunocytochemical markers

	Mean age (yr)	
	Positive	Negative
Carcinoembryonic antigen	39.1	42.1
Low-molecular-weight cytokeratin 8	39.2	44.5
High-molecular-weight cytokeratin	37.7	40.7
Vimentin	37.0	40.2
Epithelial membrane antigen	39.4	47.2

An atypical vaginal cytologic result was present in 38 instances (69.1%).

A gross lesion was present in 35 (64.6%) of the patients, whereas in 20 instances (36.4%) no detectable gross lesion was present. The diameter of the primary lesion was >2 cm in 30 patients (54.55%) and was ≤2 cm in 24 (43.64%). In one patient the size of the initial lesion could not be determined.

An improved outcome was observed in patients with invasion less than one half of the cervical stroma. The majority (24/25), including one patient with positive nodes, survived with no evidence of disease. Only one patient had recurrent disease. Patients with deep cervical invasion (greater than the inner half) experienced poor outcomes; eight of the 16 (50%) died of cancer, and lymph node metastases were observed in four of 16 (25%).

The depth of invasion was more accurately determined in the subgroup of 41 patients treated by radical hysterectomy. Twenty-five of these 41 cases had invasion of less than one half the cervical stroma, whereas the remaining 16 had deeply penetrating tumors. Disease had extended beyond the cervix in four instances.

The histologic type was designated as grade 1 in 27 (49%) of the patients, grade 2 in 14 (25.5%), and grade 3 in 14 (25.5%). Nodal metastases were detected in 10 patients (18.28%) of the entire group and in five (12%) of those treated by radical hysterectomy.

Squamous cervical intraepithelial neoplasia was also present in association with the primary adenocarcinoma in 12 patients. Six of these were associated with adenosquamous carcinomas. Two of them had positive nodal metastases, and one of these had a recurrence, resulting in death. In contrast, 25 patients had no evidence of cervical intraepithelial neoplasia. Two were initially diagnosed with adenosquamous carcinoma and three had positive lymph node metastases; six died of cancer.

The median follow-up of patients was 56 months, with a range of up to 120 months. One patient has been lost to follow-up shortly after a radical hysterectomy was performed for stage I disease and one patient died of a massive pulmonary embolus after surgery. Of the remainder, 16 (30%) are dead of disease and 37 (70%) were free of disease at the time of last examination. Twenty-two percent of those treated by radical hysterectomy failed to survive. Recurrent disease proved to be a most ominous finding. Of 17 patients in whom such a recurrence developed, only one is still alive and free of disease after a total pelvic exenteration for a central pelvic recurrence.

Immunocytochemistry. Reactivity of <10% of the tumor cells was considered negative. Most of the cases designated as positive with the various antibody markers showed immunoreactivity in >50% of the tumor cells.

For the anticytokeratin, carcinoembryonic antigen, and epithelial membrane antigen studies immunostaining of the normal squamous and glandular epithelium served as an additional internal control. No such reactivity was present in four cases. An additional four cases were nonreactive with antivimentin antibodies in the connective tissue and vascular endothelium. Either no normal tissues were present on the slides in these cases or the tissues present were nonreactive. In one case anticytokeratin antibody 35βH11 and antivimentin were not demonstrated because the slides contained no tumor cells, either because of loss of the tumor tissue during processing procedures or the actual absence of tumor cells.

Table I is a summary of the results of various antibody studies. There was no significant difference in the frequency of positive staining within the control groups with anticytokeratin 35βH11 and 34βE12. Eighty-seven percent of cases were positive with antibodies to 35βH11 but only 28% with antibodies to 34βE12. With antivimentin the corresponding tumor cells reacted in only one instance. The tissue diagnosis in this single case was poorly differentiated papillary variant of cervical adenocarcinoma without endometrial involvement. It behaved as a very aggressive neoplasm. Carcinoembryonic antigen was positive in 92% of the control slides but in only 67% of the tumor tissue.

Table II looks at the immunocytochemical results as a function of patient age. Although there were no significant differences between the two groups, patients with a negative staining reaction were consistently older than those with a positive reaction. This difference was

Table III. Immunocytochemical analysis of histologic types of cervical adenocarcinoma

	Anti-35βH11				*Anti-34βE12*				*Antivimentin*				*Anti-carcinoembryonic-antigen*			
	Positive		*Negative*		*Positive*		*Negative*		*Positive*		*Negative*		*Positive*		*Negative*	
Histologic diagnosis	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
Adenocarcinoma	38*	86	6	14	12	29	30†	71	0		42†	100	30	70	13	30
Adenosquamous carcinoma	9	90	1	10	3	30	7	70	0		10	100	6	60	4	40
Mesonephric adenocarcinoma	NT		0		0		1		0		1		0		1	
Papillary serous carcinoma	1		0		0		1		1		0		1		0	
TOTAL	48		7		15		39		1		53		37		18	

NT, No tumor present on slides.

*Two with no tumor.

†One with no tumor.

Table IV. Tumor size and immunocytochemical staining results ($N = 54$)

	Tumor size ≤2 cm		*Tumor size >2 cm*		*Total*	
	Positive	*Negative*	*Positive*	*Negative*	*Positive*	*Negative*
35βH11, low-molecular-weight cytokeratin	20	2	25	5	45*	7
34βE12, high-molecular-weight cytokeratin	9	14	6	24	15†	38
Carcinoembryonic-antigen‡	12	12	24	6	36	18
Epithelial-membrane-antigen	22	2	27	3	49	5

*No tumor in two cases.

†No tumor in one case.

‡$p < 0.01$.

particularly apparent in epithelial membrane antigen (47.2 vs 39.4 years).

Table III details the distribution of positive staining to immunocytochemical markers with respect to the histopathologic diagnosis. The majority of cases were diagnosed merely as adenocarcinoma. Ten patients had adenosquamous carcinomas. One was defined as an adenocarcinoma of mesonephric origin and another as a papillary serous carcinoma. Forty-four of the 55 patients (80%) had stage IB lesions. The numbers in clinical stages beyond IB were too small to permit a statistical evaluation, but no significant differences in tissue reactivity could be seen between the various stages.

Table IV is a comparison of tumor size and the antigenic profile of the tumor in 54 patients; tumor size was designated as >2 or ≤2 cm in maximum dimension. Although the presence of epithelial membrane antigen and the various cytokeratins proved to be of no significant difference between the two groups, carcinoembryonic antigen expression was present in only 50% of patients with small tumors but in >75% of cases with tumors >2 cm. This difference is significant with $p < 0.01$.

Table V is a comparison of immunocytochemical markers in patients who survived without evidence of disease and those who died of disease. One patient was lost to follow-up and another died postoperatively. Expression of carcinoembryonic antigen was demonstrated in 11 of 16 patients who died of disease and in 26 of 37 who have no evidence of disease.

The 41 patients treated surgically by radical hysterectomy and bilateral lymphadenectomy represent a large and relatively homogeneous group with many similar clinical characteristics. They may be appropriately divided into two groups; group 1 consisting of 21 patients whose tumor measured ≤2 cm and group 2 consisting of 20 patients whose tumor diameter was >2 cm. In each group the response to the immunocytochemical markers together with several clinical pathologic factors is summarized in Table VI. The difference in expression of carcinoembryonic antigen between the two groups does approach statistical significance ($p = 0.058$). Group 1 clearly demonstrated an improved outlook. The majority of these patients (18/21) had grade 1 lesions, although five had the adenosquamous variant. Although no patient in this group had lymph node metastases, two of the 21 did have recurrence. The remainder (19/21) clinically have no evidence of disease. Among the patients in group 2 with larger lesions, 45% showed grade 3 histologic type, five of the 20 (25%) had lymph node metastases, and recurrent disease developed in seven instances (35%).

Table V. Status versus immunocytochemical results

Status	No.	*Low-molecular-weight cytokeratin (n = 52)*		*High-molecular-weight cytokeratin (n = 53)*		*Carcinoembryonic antigen (n = 54)*		*Vimentin (n = 53)*		*Epithelial membrane antigen (n = 54)*	
		Positive	*Negative*	*Positive*	*Negative*	*Positive*	*Negative*	*Positive*	*Negative*	*Positive*	*Negative*
No evidence of disease	37	30	5	10	26	26	11	0	36	33	4
Dead of disease	16	15	1	4	12	11	5	1	15	15	1
Dead of concomitant disease	1	0	1	1	0	0	1	0	1	1	0

Two of the patients with nodal metastases remain free of disease, but it is noteworthy that among the nine cases of recurrent carcinoma four patients had negative lymph nodes.

Comparable data were observed in patients with a depth of invasion greater than or lesser than one half the depth of the cervical stroma. Twenty-five of 41 patients demonstrated depth of invasion of less than one half of the cervical stroma with carcinoembryonic antigen–positive reactivity in 17 instances (68%). Anticytokeratin 34βE12 stained positively in nine patients. In 16 patients the depth of invasion exceeded the inner one half of the cervical stroma, and positive carcinoembryonic antigen was expressed in 10 cases (62.5%). This included four patients in whom the tumor extended beyond the cervix. All of these cases were positive for carcinoembryonic antigen.

Sixteen patients who used oral contraceptives before the diagnosis of adenocarcinoma were compared with 13 patients who denied any contraceptive practice. The patterns of carcinoembryonic antigen immunostaining were significantly different between the two groups. The tumors showed carcinoembryonic antigen expression in 14 of the 16 using oral contraceptives although in only seven of the 13 who denied contraceptive use. This difference is statistically significant ($p = 0.04$), although the explanation is not readily apparent. Moreover, in the group that used oral contraceptives only one of the 16 had nodal metastases and the survival rate was 86% (12/14). Among those who did not use contraception there were six instances of nodal disease and the corresponding survival rate was only 61% (8/13).

Comment

Several investigators have confimred the value of immunocytochemistry in distinguishing between primary adenocarcinoma of the cervix and stage II adenocarcinoma of the endometrium with cervical extension. Vimentin expression by endometrial adenocarcinoma but not by cervical adenocarcinoma is now recognized as the most reliable marker in resolving this confusion.[9]

Table VI. Summary of clinicopathologic and immunocytochemical findings in 41 patients treated by radical hysterectomy

	Disease ≤2 cm (n = 21)	*Disease >2 cm (n = 20)*
Positive immunocytochemical marker		
Low-molecular-weight cytokeratin	19 (90%)	17 (85%)
High-molecular-weight cytokeratin	9 (43%)	3 (15%)
Epithelial membrane antigen	20 (95%)	19 (95%)
Carcinoembryonic antigen	11 (52%)	16 (80%)
Pathologic factor		
Grade 3	2 (9.5%)	9 (45%)
Adenosquamous carcinoma	5 (24%)	3 (15%)
Positive nodes	0	5 (25%)
Clinical factor		
Recurrence	2 (9.5%)	7 (35%)
No evidence of disease	19 (90%)	12* (60%)

*One death after postoperative massive pulmonary embolus.

There is, however, very limited information available to use on long-term follow-up of patients with cervical adenocarcinoma analyzed by contemporary immunohistochemical staining with a panel of markers.

Wahlstrom et al.[14] have reported on differences in the distribution of anti–carcinoembryonic antigen immunostaining between adenocarcinoma of the endometrium and endocervix. Endometrial adenocarcinoma was positive in only 8% of the cases stained, whereas endocervical adenocarcinoma was positive in 80%. Our observation that 69% of patients with cervical adenocarcinoma expressed carcinoembryonic antigen correlates well with the findings of others.[8, 15] The correlation between tumor size and positivity of carcinoembryonic antigen immunostaining is also of interest. Van Nagell et al.[16] have reported on plasma determination values of carcinoembryonic antigen in patients with cervical cancer. They concluded that the absolute carcinoembryonic antigen level appears related to tumor burden as measured by tissue concen-

tration and the extent of disease. There was a direct correlation with recurrent cancer in >80% of their patients. However, the analysis of Van Nagell et al. was derived predominantly from patients with squamous cell carcinoma. In 25 patients with cervical adenocarcinoma, only nine (36%) proved to have positive results for carcinoembryonic antigen. Our findings do suggest that patients with carcinoembryonic antigen expression are at an increased risk for recurrence and also sustain a reduced survival potential. Among such patients serial carcinoembryonic antigen plasma values might be of value in the effort to detect recurrent disease at an early stage. It should be emphasized that the level of reactivity in immunologic staining reflects the extent of cellular proliferation rather than merely the presence of malignancy.

Several studies have confirmed the significance of lymph node metastases as a major risk factor for recurrence in cervical cancer.[17-20] Our report also observed that six of nine patients with recurrent stage IB disease had no evidence of nodal spread as determined by conventional microscopic assessment. Immunocytochemistry has been used to detect micrometastases in bone marrow among patients with breast[21] and ovarian[22] cancers, and immunocytochemical staining in the bone marrow has proved predictive of recurrence in such patients. Immunocytochemistry has improved detection of axillary lymph node micrometastases in patients with breast cancer. It would seem reasonable to suggest that the same technology might be appropriate in adenocarcinoma of the cervix and that we should not restrict our approach to conventional microscopy. Among those patients with carcinoembryonic antigen–positive tumors, carcinoembryonic antigen immunostaining of the lymph nodes might reveal otherwise unsuspected nodal spread and serve as a foundation for adjunctive therapy in an effort to minimize the incidence of local or regional failure.

One of the current challenges in gynecologic oncology is to distinguish those patients at high risk for tumor recurrence and to detect such recurrent disease at an earlier time. It is quite possible that in the future the use of a panel of various tumor markers might allow us to distinguish such a group of patients who would benefit from additional observation, surveillance, or therapy. Serial monitoring of serum in the postoperative, postirradiation, and postchemotherapy phases of patient treatment might prove a significant addition to our pattern of surveillance. Certainly, at the present time carcinoembryonic antigen would appear to be an excellent candidate.

The substantial mortality among patients with recurrence of cervical adenocarcinoma emphasizes the need for continuing research into the epidemiologic characteristics, pathogenesis, presentation, and therapy of this disease. Success in achieving control over cancer must finally depend on our ability to shed light on the natural history of malignancy. It is quite possible that the use of serial markers, particularly the more recently acquired agents, may help us to understand the evolution of endocervical columnar atypia[23] or dysplasia or to define the biologic behavior of endocervical glandular tumors of the so-called minimal deviation adenocarcinoma.[24-26] Immunocytochemistry can offer us an additional tool for such investigations and should be exploited in every direction.

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Discussion

DR. GLEN E. HAYDEN, Seattle, Washington. Several important clinical points require emphasis. Vigorous endocervical curettage is a necessary step in the diagnosis of grade 2 cervical intraepithelial carcinoma because adenocarcinoma of the cervix is frequently associated with it. Cytologic screening for early diagnosis of cervical adenocarcinoma is not highly effective. The authors' data indicate that outcome closely follows the depth of invasion; when less than one half of the endocervix is involved, the outcome is favorable. Interestingly, patients using oral contraceptives had a better outcome regardless of the stage. Oral contraceptives also reduce the incidence of endometrial carcinoma, and in that disease the outcome is improved if the patient has been receiving estrogen therapy.

The most important part of this presentation is the lack of expression of vimentin in 54 of 55 lesions of the endocervix. The authors' ability to distinguish between endocervical and endometrial carcinoma was well demonstrated. The single specimen with the positive monoclonal antibody test result for vimentin has several possible explanations; endometrial cell contamination is the more likely and the rare endometrioid lesions, either primary or secondary from the ovary, is the less likely.

Anti–carcinoembryonic antigen immunostaining also predicts outcome for endocervical lesions, particularly the more extensive lesions. The presence of carcinoembryonic antigen staining strongly suggested a poorer prognosis. Immunocytochemistry may be of value in the future as an additional method for surveillance and monitoring of therapy.

DR. WILLIAM A. PETERS III, Seattle, Washington. It is often difficult to distinguish a stage I adenocarcinoma of the cervix from a stage II carcinoma of the endometrium. The technique that we have come to rely on in making this distinction is magnetic resonance imaging which has been reliable although quite expensive. The vimentin antibody test is a more direct way of getting the information and may eliminate the need for magnetic resonance imaging in many patients.

We are seeing increased numbers of patients with adenocarcinoma in situ, and the distinction from early invasive adenocarcinoma of the cervix is frequently very difficult to make. Dr. Tamimi, have you looked at specimens from patients with adenocarcinoma in situ and from patients with early stromal invasion? If so, have you found immunocytochemistry to be of any benefit in making the distinction?

DR. PHILIP J. DISAIA, Irvine, California. Carcinoembryonic antigen, a fetal oncogene, is probably expressed by the mechanism of derepression. Did you correlate carcinoembryonic antigen with tumor grade? Did you see it more commonly in high-grade tumors? Dr. Peters has alluded to the possibility of using carcinoembryonic antigen as a diagnostic tool to differentiate adenocarcinoma of the endometrium that secondarily involves the cervix rather than adenocarcinoma of the cervix that involves the endometrium. How quickly can you do this test? Is it really available within days so that it is useful to the clinician?

DR. WILLIAM LUCAS, Ashland, Oregon. We at the University of California, San Diego, like many other oncology groups around the world, started to notice an increase in the incidence of adenocarcinoma relative to other histologic types of cancer of the cervix in the early 1980s. When there appears to be a significant shift in histologic types, it seems to me that some environmental factor is operating. Do you have any ideas about epidemiologic studies that might identify those factors so that we would perhaps have a better chance at prevention? Dr. Hayden referred to the possible protective effect of oral contraceptives. I wonder if the conclusion might be premature until we make sure that endocrine factors are not operative in increasing the incidence of adenocarcinoma of the cervix. Although cervical adenocarcinoma associated with oral contraceptives may be a more benign disease, as is estrogen-associated endometrial adenocarcinoma, we need to be sure that an analogous situation does not exist for inducing cervical adenocarcinoma.

DR. TAMIMI (Closing). Dr. Hayden asked whether the finding of a positive vimentin stain in the single patient was contamination or an error in diagnosis. I do not think that it was an error in diagnosis because we looked at this case carefully in terms of the histologic type and clinical course. It was a poorly differentiated adenocarcinoma of the cervix limited to the cervix and was

not an endometrial cancer. Thus this was a positive vimentin test in an adenocarcinoma of the cervix. There have recently been reports of coexpression of vimentin in breast cancers.[1-3] These investigators reported on very anaplastic tumors and considered vimentin to be a prognostic marker. We think that positive vimentin coexpression indicates an anaplastic tumor with a very aggressive clinical course. We happen to have seen only one case so far, but I suspect that as we continue to look for it we will find more.

Dr. Peters, we looked at the immunochemical results of invasive adenocarcinoma only, not adenocarcinoma in situ.

Dr. DiSaia, you asked why the carcinoembryonic antigen level is high in our subjects. This finding does not differ from that in other studies. For example, Wahlstrom et al.[4] reported an 80% incidence of positive carcinoembryonic antigen. You also asked about the time it takes to obtain results after the immunocytochemistry tests. Within 3 days of the request we obtain results of cytokeratin, vimentin, and carcinoembryonic antigen. For patients with malignancy we are now also looking at oncogenes, P53, and growth factors.

Dr. Lucas, I do not know whether oral contraceptives are related to the increased incidence of cervical adenocarcinoma. Patients who are exposed to oral contraceptives and have adenocarcinoma of the cervix tend to have had long-term exposure, 5 to 10 years. To answer this question will require in-depth epidemiologic studies.

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Advanced epithelial ovarian carcinoma: Long-term survival experience at the community hospital

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Santa Rosa, California

OBJECTIVE: The purpose of this study was to determine prognostic factors that could be altered to increase survival of patients with advanced ovarian cancer treated at the community hospital.
STUDY DESIGN: This study is a retrospective analysis of 101 patients with stage III and IV epithelial ovarian carcinoma who underwent primary surgery at two private hospitals from 1970 to 1990.
RESULTS: Primary laparotomy was done by a general surgeon in 54 cases. The tumors in 23% of stage IIIC cases were debulked to ≤2 cm residuum. The survival rate decreased as stage or postoperative tumor residual increased. Patients with stage IIIA and IIIB disease had similar survival rates, significantly better than those with stage IIIC disease. Cytoreduction of stages IIIB and IIIC to microscopic disease resulted in a survival curve equal to the "natural optimal" stage IIIA. Patients with cytoreduction of stage IIIC disease to ≤2 cm did not obtain survival rates equal to those with stage IIIB disease with visible tumor remaining. Patients with stage IIIC disease achieved a significant increase in survival rate if their tumors were cytoreduced to microscopic disease. Platinum-based combination chemotherapy compared with alkylating agents improved survival in patients with stage IIIC disease who had >2 cm tumor residual. Seven patients survived >5 years, with three patients currently free of disease. Three prognostic categories predict decreasing survival: (1) stage IIIC if tumor is cytoreduced to no visible residual, stage IIIA or stage IIIB; (2) stage IIIC with visible tumor residual; (3) stage IV.
CONCLUSION: Survival of community hospital–treated advanced ovarian carcinoma patients can be improved by early diagnosis, adjuvant platinum-based combination chemotherapy, and surgical cytoreduction to minimal disease. This treatment requires a team approach and education of the medical staff, including nongynecologists. (Am J Obstet Gynecol 1992;166:1663-72.)

Key words: Ovarian carcinoma, survival rate, chemotherapy, residual tumor

Ovarian cancer is a devastating disease. It was estimated that in 1991 there would be 20,700 new cases of ovarian cancer in the United States. This represents 29% of all new cases of genital organ cancer in females. Most patients present with advanced stage disease, and prognosis is poor. For 1991 12,500 deaths from ovarian cancer are estimated; this amounts to 53% of the expected deaths from all female genital cancer, placing ovarian cancer as the fifth most common cause of cancer death among women in the United States.[1]

Staging categories for primary carcinoma of the ovary are based on findings at surgical exploration. In 1985 the Oncology Committee of the International Federation of Gynecology and Obstetrics (FIGO) changed the definition of stage III cancer of the ovary. Stage III is now subdivided into categories A, B, or C on the basis of the size of the peritoneal implants outside the pelvis or positive retroperitoneal or inguinal lymph nodes (Table I).[2]

It was believed that a retrospective study of advanced-stage epithelial ovarian carcinoma in a suburban northern California city could give useful information concerning patients who are operated on in local community hospitals. Long-term survival and prognostic categories could be refined by the new staging system and added to the literature of ovarian cancer derived in the United States, mostly from referral university medical centers. It has been suggested that specialist cancer units acting as tertiary referral centers may well attract a biased population.[3]

Material and methods

Cases were chosen from two private hospitals in Santa Rosa, California, Santa Rosa Memorial Hospital and Warrack Hospital, for the period Jan. 1, 1970, to Dec. 31, 1990 (records for the first 2 years of the study were not available from Warrack Hospital). The city of Santa Rosa is 50 miles north of San Francisco; it had a population of 50,000 in 1970 (14.8% ≥65 years of age) and 112,000 in 1990 (16% ≥65 years of age). Santa Rosa Memorial Hospital is a 225-bed, not-for-profit, religious-sponsored hospital that had 220,415 inpatients

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6/6/37493

Table I. FIGO definition of stage III and IV carcinoma of the ovary

Stage III.	Tumor involving one or both ovaries with peritoneal implants outside the pelvis and/or positive retroperitoneal or inguinal nodes. Superficial liver metastasis equals stage III. Tumor is limited to the true pelvis, but with histologically verified malignant extension to small bowel or omentum.
Stage IIIA.	Tumor grossly limited to the true pelvis with negative nodes, but with histologically confirmed microscopic seeding of abdominal peritoneal surfaces.
Stage IIIB.	Tumor of one or both ovaries, with histologically confirmed implants of abdominal peritoneal surfaces, none >2 cm in diameter. Nodes negative.
Stage IIIC.	Abdominal implants >2 cm in diameter and/or positive retroperitoneal or inguinal nodes.
Stage IV.	Growth involving one or both ovaries with distant metastasis. If pleural effusion is present there must be positive cytologic test results to allot a case to stage IV. Parenchymal liver metastasis equals stage IV.

Staging is based on findings at clinical examination or surgical exploration.

(excluding normal newborns) during the study period. Warrack Hospital is a 79-bed, acute-care, for-profit hospital that had 59,560 inpatients during the period Jan. 1, 1972, to Dec. 31, 1990. The hospitals are 1 mile apart and have similar surgical and medical oncology staffs. There is no gynecologic oncologist immediately available in the area; medical oncologists supervise chemotherapy treatment.

The inpatient discharge diagnosis list was reviewed for the study years for the two hospitals. Three-hundred-forty patients were coded as 1830 International Classification of Diseases–Clinical Modification (primary malignant neoplasm, ovary). 24 charts could not be located; therefore 316 charts were reviewed. The criteria for inclusion in the study required the patient to have stage III or IV primary epithelial ovarian carcinoma diagnosed by initial laparotomy at either hospital during the above study period. Two hundred fifteen cases did not meet the criteria. One case of primary peritoneal carcinoma was excluded. Not included in the analysis are 103 patients who had initial surgery elsewhere, 19 patients with presumed diagnosis of ovarian cancer but who did not have surgery, 2 patients with initial surgery before 1970, and 36 patients who did not have invasive primary epithelial ovarian adenocarcinoma. Of the 103 other hospital cases, 12 were noted to have had primary surgery by referral from Santa Rosa to a medical center. The 155 cases of primary epithelial ovarian cancer operated on at the study hospitals were staged by retrospective chart review. Forty-seven were stage I and seven were stage II (note that only three of these stage II had omental biopsies). The 101 cases staged as III and IV comprise the basis for this report, representing 65% of the primary ovarian epithelial carcinomas. Seventy-seven patients had initial surgery at Memorial Hospital and 24 at Warrack. When appropriate, cases were discussed with the primary surgeon or primary care physician and available outpatient records were reviewed. Residual tumor after primary surgery was coded by the diameter of the largest remaining nodule and subdivided into nonvisible (microscopic), ≤2 cm, or >2 cm. Estimation of residual tumor from the operative note was not possible in one stage IIIC patient operated on in 1970 who survived 4 months after receiving postoperative irradiation. Data were also gathered on potential independent prognostic factors as follows: postoperative adjuvant therapy, age of patient at primary surgery, parity, speciality of primary and assistant surgeons, histologic characteristics and grade of tumor, ascites, and whether the patient had had a prior hysterectomy. Patient performance status as a prognostic variable could not be evaluated because the hospital charts did not contain sufficient information to retrospectively assign a Karnofsky index. Percentages are not given for occurrences related to the entire patient population because the number of cases will essentially equal percent if the denominator is 101.

Survival curves were prepared with the Kaplan-Meier method.[4] Survival was defined as months from primary surgery to date of death. Tic marks were placed on the survival curves to represent the follow-up times of patients alive as of Dec. 31, 1990. Deaths were checked with local death certificates, and all patients who died did so with disease. Results are reported as 5-year and median survival rates.

The log-rank test was used to compare survival curves by obtaining a χ^2 value. Statistical significance was considered at $p < 0.05$. When categories of a prognostic variable followed an expected ordering such as stage, a test for linear trend of survival was used to report the overall significance of that variable. Analysis for the χ^2 was done within prognostic strata when appropriate according to the method of Peto et al.[5]

Results

The median age at primary surgery was 65 years 6 months (mean age 64 years 2 months), with a range from 33 years 5 months to 88 years 10 months. There were 9 patients <45 years of age, 36 >70 years, and 19 patients >75 years. No significant difference in survival curves could be demonstrated for age groupings even if divided at 5-year intervals from 45 to 75 years.

Review of the operative findings at primary surgery

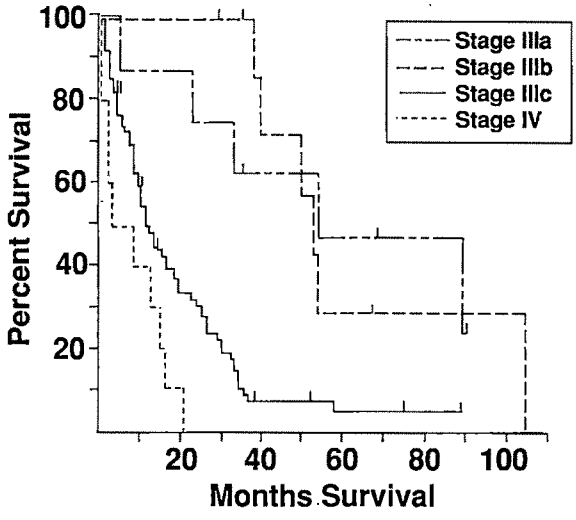


Fig. 1. Survival by FIGO stage. Chi-square test for trend yielded 21.2; $p < 0.001$. p values for individual curves: IIIA vs IIIB, not significant; IIIB vs IIIC, 0.001; IIIC vs IV, <0.025.

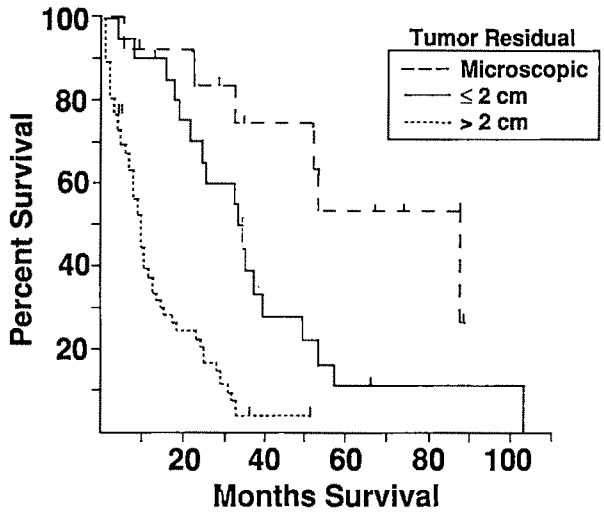


Fig. 2. Survival according to the largest tumor residual after primary surgery in patients with stage III disease. X^2 for trend yielded 28.8; $p < 0.001$.

according to the latest FIGO staging classification yielded 8 stage IIIA, 9 stage IIIB, 74 stage IIIC, and 10 stage IV cases. Survival according to stage is shown in Fig. 1. Five-year/median survival rates are as follows: stage IIIA, 47%/50 months; stage IIIB, 29%/51 months; stage IIIC, 5%/11 months; stage IV, 0%/3 months. Fig. 2 shows survival curves for stage III patients based on the tumor left in the abdomen after primary surgery. Five-year/median survival rates are as follows: microscopic (n = 13), 53%/58 months; ≤2 cm (n = 21), 11%/34 months; >2 cm (n = 56), 0%/9 months.

Initial surgical procedures are listed in Table II. Only 43 patients had complete removal of uterus, tubes, and ovaries. Bowel surgery included 4 small bowel resections, 2 large bowel resections, 3 internal bowel bypass procedures, and 2 colostomies. In six cases the abdominal incision was a low transverse Pfannenstiel. No patient had pelvic or periaortic lymph node sampling. Primary surgery resulted in the debulking of stage IIIC tumors to ≤2 cm in 23% (17 cases) for the entire series. For the years 1970 to 1979 debulking to ≤2 cm nodules was accomplished in 14% for stage IIIC tumors; for the years 1980 through 1990 this figure rose to 32%. In stage IIIC patients survival for the years 1980 through 1990 was better than for the period 1970 through 1979 (Fig. 3). Five-year/median survival rates increased from 0%/9 months to 10%/19 months, a significant difference at $p < 0.005$. The death-rate ratio of 0.48 showed that stage IIIC patients were dying at half the rate in the later years.

Six of the 101 patients died postoperatively. Table III lists the characteristics of these six patients. None had radical surgery; all six appeared to have died from the disease or predisposing cardiac factor and not from a direct complication of the surgery.

The specialty of the primary surgeon was listed as general surgeon in 54 cases, gynecologist in 46 cases, and urologist in 1 case initially seen with ureteral obstruction. In stages IIIC and IV cases the primary surgeon was a general surgeon in 48 (58%) cases and a gynecologist in 34 (40%) cases. In 23 (27%) of the 84 stage IIIC and IV cases both a gynecologist and general surgeon operated. The reverse was found for the earlier stage IIIA and IIIB cases, in which the primary surgeon was a gynecologist 71% of the time and a general surgeon 29% of the time. Both a gynecologist and a general surgeon operated together in 5 (29%) of the 17 cases of stages IIIA and IIIB disease. There was no significant difference in survival with respect to surgical specialty.

Eighty-nine patients had received postoperative adjuvant therapy. In nine cases it could be determined that no adjuvant therapy was given, and in three cases it was unknown. Three patients were irradiated and 86 received chemotherapy. Treatment with chemotherapy was divided into 59 patients who received platinum-based combination chemotherapy and 27 patients primarily treated with alkylating agents. Treatment with cisplatin (Platinol) was initiated in 1978. Cisplatin dosage was 50 mg/m²; it was used in combination with cyclophosphamide (Cytoxan) and doxorubicin (Adriamycin) until November 1987, when doxorubicin was deleted. Carboplatin (Paraplatin) has been substituted for cisplatin since 1990. The dose of carboplatin is 300 mg/m²; the combination with cyclophosphamide (600

Table II. Procedures performed at primary operation

Procedure	No. of patients	FIGO tumor stage			
		IIIA	IIIB	IIIC	IV
Pelvic organs removed					
TAH-BSO or BSO* or USO† and ICO	34	5	6	21	2
TAH-BSO or BSO* or USO† (no ICO)	9	2	2	4	1
Pelvic organs incompletely removed					
BSO and ICO	8	0	0	7	1
USO and ICO	3	0	0	3	0
BSO	3	0	1	1	1
USO	9	1	0	6	2
ICO	7	0	0	6	1
Biopsy	28	0	0	26	2
TOTAL	101	8	9	74	10

TAH-BSO, Total abdominal hysterectomy and bilateral salpingo-oophorectomy; *BSO,* bilateral salpingo-oophorectomy; *USO,* unilateral salpingo-oophorectomy; *ICO,* infracolic omentectomy.

*Patients with prior hysterectomy.

†Patients with prior hysterectomy and USO.

Table III. Characteristics of the six patients who died during initial hospitalization

Year	Postoperative day	Stage	Surgery	Age	Diagnosis
1974	3	III C	Biopsy	68	Hypovolemic shock
1988	9	III C	Omentectomy	67	Congestive heart failure*
1980	11	III C	Biopsy	82	Congestive heart failure
1984	15	III C	USO	75	Malnutrition
1973	11	IV (liver)	Biopsy	72	Bowel obstruction
1978	29	IV (lung)	BSO and colostomy	37	Sepsis

All patients had marked abdominal carcinomatosis. *USO,* Unilateral salpingo-oophorectomy; *BSO,* bilateral salpingo-oophorectomy.

*Patient with severe atherosclerotic cardiovascular disease.

mg/m²) is currently the protocol for initial adjuvant chemotherapy by the medical oncologists. All three of these platinum-based combinations were calculated together as platinum chemotherapy because other studies have shown the three combinations to be similar.[6-8] Until 1978 there were varied uses of melphalan (Alkeran), cyclophosphamide, and chlorambucil (Leukeran) as primary adjuvant chemotherapy. In the early years of this study six patients also received intraperitoneal triethylenethiophosphoramide (Thiotepa). Salvage therapy for progressive disease was varied with no set protocol. In recent years 10 patients with persistent intraabdominal disease have received intraperitoneal instillation of cisplatin and etoposide.

In all cases treated with chemotherapy, platinum contributed to increased survival (Fig. 4, graph A). Five-year/median survival of platinum-based combination chemotherapy ($n = 59$) versus alkylating agents ($n = 27$) were 14.9%/24 months versus 4.3%/10 months, with a death-rate ratio of 0.58. However, comparison of subgroup survival curves for platinum-based chemotherapy versus alkylating agents did not show statistically significant differences in stages IIIA, IIIB, and IV, or IIIC when tumor residual after initial surgery was ≤2 cm. Platinum-based combination chemotherapy did increase survival in the stage IIIC patients who had tumor residual >2 cm (Fig. 4, graph B). The death-rate ratio of 0.46 illustrates that platinum-based combination chemotherapy ($n = 26$) prevented or delayed about 50% of the deaths that would occur with alkylating agents ($n = 19$); median survival was 15 months compared with 9 months, but there were no long-term survivors.

The histologic characteristics and grade of the tumors are listed in Table IV. No difference in prognosis could be found in histologic characteristics or tumor grade. Combination of the two, as suggested by Dembo and Buch[9] also did not demonstrate any relationship to survival.

Sixty-four patients were recorded to have ascites at the primary surgery. Twenty-six patients had no ascites, and in 11 cases the presence of ascites was not stated. There was no significant difference in survival whether ascites was present or not.

There were 12 nulliparous women in the series. In 16 cases the parity was unknown. There was no difference in survival between parous and nulliparous patients.

Review of the medical records revealed that 35 patients had had prior hysterectomies without removal of

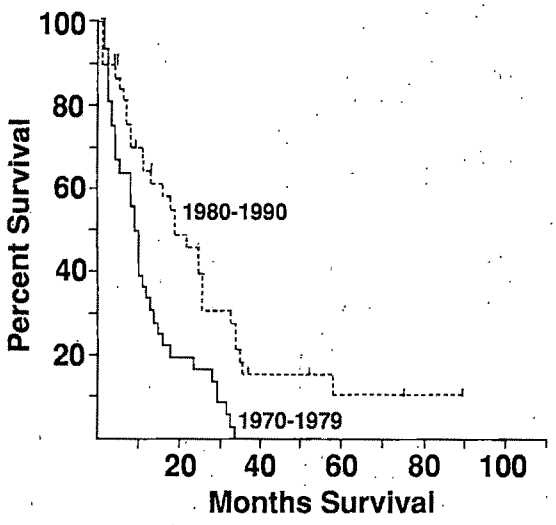


Fig. 3. Survival according to the years 1970 through 1979 (n = 36) compared with 1980 through 1990 (n = 38) for patients with stage IIIC disease. $\chi^2 = 9.6$; $p < 0.005$.

both ovaries. No difference in survival was noted for those with or without prior hysterectomy.

The presenting symptoms that most frequently brought the patient to a physician are seen in Table V. Symptoms are predominantly abdominal and not specifically pelvic in most cases. Five patients sought medical advice for abdominal masses they palpated themselves and five others for inguinal masses. Preoperative diagnoses for other abdominal conditions included bowel obstruction, 6 patients; inguinal hernia, 2; diverticulitis, 2; bowel adenoma, 1; and umbilical, ventral, and hiatal hernias, 1 each.

A pelvic mass found at a routine gynecologic examination led to surgery in six patients. Of the six, only one stage IIIC patient with tumor residual >2 cm died after 7 months' survival. The other five patients are still alive at 35 months (stage IIIA), 35 and 67 months (stage IIIB), and 37 and 89 months (stage IIIC). One patient was diagnosed from a routine Papanicolaou smear that showed adenocarcinoma cells. One patient who did not have a palpable pelvic mass was operated on by urologists for ureteral obstruction.

The characteristics of the seven 5-year survivors are shown in Table VI, which highlights only three long-term survivors with no evidence of disease. Six other patients are alive with no evidence of disease representing stages and residual disease as follows: IIIC, >2 cm (52 months); IIIA, (35 months); IIIB, ≤2 cm (35 months); IIIB, microscopic disease, (29 months); IIIC, ≤2 cm (13 months); and IIIC, microscopic disease (9 months).

A significant relationship between the size of residual tumor and survival rate was demonstrated for patients with stage IIIC platinum-treated disease (Fig. 5). The 5-year/median survival rates for residual disease subgroups were as follows: microscopic disease, 100%/median not yet reached (n = 3); ≤2 cm, 0%/25 months (n = 11); >2 cm, 0%/15 months (n = 26). If only tumor residual ≤2 cm is compared with the subgroup with >2 cm residual, χ^2 is 1.32 yielding $p < 0.3$ and >0.2, which is not significant.

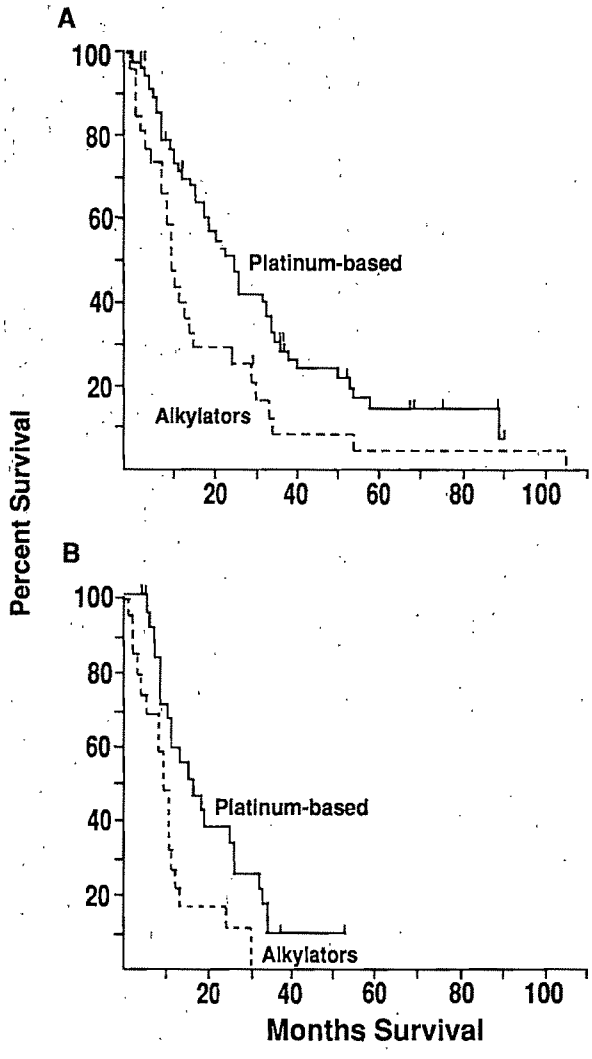


Fig. 4. Survival according to adjuvant chemotherapy **A.** All 86 patients; $\chi^2 = 5.1$; $p < 0.025$ **B.** Patients with stage IIIC disease with >2 cm residual disease; $\chi^2 = 6.3$; $p \simeq 0.01$.

Five patients' tumors were cytoreduced from stage IIIB (n = 2) and IIIC (n = 3) to no visible tumor residual remaining (microscopic). The tumors of 13 stage IIIC patients treated with adjuvant chemotherapy were cytoreduced to ≤2 cm residuum. Table VII indicates that cytoreduction of stage IIIB and IIIC tumor to no visible tumor at the finish of primary surgery results in long-term survival rates comparable with stage IIIA. However, stage IIIC patients with tumors debulked to ≤2 cm were not able to obtain survival curves as good as stage IIIB patients left with visible tumor.

Table IV. Histologic characteristics and tumor grade

Histologic characteristics	*Tumor grade*				
	1	*2*	*3*	*Ungraded*	*Total*
Serous	3	17	17	12	49
Mucinous	0	3	4	5	12
Endometrioid	0	1	5	1	7
Clear cell	1	1	0	1	3
Undifferentiated	0	2	20	6	28
Combination	0	2	0	0	2
TOTAL	4	26	46	25	101

Table V. Presenting symptoms

Symptom	*Number*	*Average duration*
Abdominal		
Pain	58	2½ mo
Distention	48	2 mo
Dyspepsia, nausea, vomiting	15	2 mo
Constipation	10	2½ mo
Diarrhea	6	5 wk
Pelvic		
Pain*	7	2 mo
Urinary difficulty	5	7 wk
Vaginal bleeding	3	2½ mo
Dyspareunia	2	2 mo
General		
Weight loss–gain	18/6	3½ mo
Anorexia	13	5 wk
Fatigue, weakness	11	4 mo

Most patients presented with multiple symptoms.
*Two of these patients also had upper abdominal pain.

Prognosis for a patient who survives primary surgery and is treated with appropriate adjuvant chemotherapy can be obtained from one of three categories based on calculations from this retrospective data. Stage IIIC patients with no visible tumor residual and stages IIIA and IIIB patients had 4- and 5-year survival rates of 67% and 45%, with a median survival rate of 54 months (n = 20); stage IIIC patients with visible tumor residual, had 4- and 5-year survival rates of 8% and 0%, with a median survival rate of 19 months (n = 39); stage IV patients had 4- and 5-year survival rates of 0% and 0%, with a median survival rate of 8 months (n = 8).

Comment

A retrospective chart review of patients with advanced ovarian adenocarcinoma who had primary surgery in a community hospital was done to delineate factors affecting long-term survival. The median age of the patients, 65 years 6 months, suggests that this community-based population could be different from the usually reported university experience. Median ages from comparative studies include 52 years,[10] 53 years,[11] 54 years,[12] and 58 years[13] (the study of Heintz et al.[13] recorded 17% of patients >70 years of age; in my study 36% were >70 years of age). The new FIGO staging classification for stage III disease predicted the subdivisions IIIA and IIIB to have significantly better prognosis than IIIC. The 5-year survival rates for stages IIIA, B, and C of 47%, 29%, and 5%, respectively, are compared with one published standard of 31%, 32%, and 14%, respectively.[14] Confirmation of the terribly poor survival of stage IV cases in spite of adjuvant therapy is the 0% at 5-year and only 8 months' median survival. It is interesting that patients with stage IIIA disease did not have a significantly better overall survival rate than stage IIIB. Incorrect staging affects a study such as this because so many incomplete initial staging procedures were done. The magnitude of this problem has been reported by others.[15-17] For example, in a Gynecologic Oncology Group study of surgical staging, 13 (22%) of 58 patients had the stage of disease elevated at reexploration by a complete surgical-pathologic search.[18] Also, not one of the 71 stage I, II, IIIA, and IIIB charts I reviewed noted retroperitoneal lymph node sampling, which if positive places the patient's disease into stage IIIC. It has been shown that a high percentage of ovarian cancer cases will have retroperitoneal pelvic and periaortic lymph nodes involved with cancer.[19] As shown in a series of 173 stage III cases, lymph node involvement was a negative prognostic factor for survival.[20] The two reasons that have been outlined as the cause for incomplete surgical staging seem applicable to our community.[21] Lack of knowledge about ovarian cancer metastases applies to the cases treated by general surgeons, and lack of surgical expertise pertains to gynecologists technically unable to perform retroperitoneal lymph node biopsies.

The importance of primary surgical cytoreduction in advanced ovarian cancer seems to be established.[22, 23] The findings of my study confirm that cytoreduction at primary surgery can be associated with improved long-term survival. Figs. 2 and 5 show that the larger the tumor residual at primary exploratory laparotomy,

Table VI. Characteristics of seven long-term (5 years) survivors

Months' survival	*Year of surgery*	*Patient status*	*Tumor stage*	*Tumor residual*	*Platinum chemotherapy*	*Histologic characteristics–tumor grade*
105	1974	DOD	III B	≤2 cm	No	Serous-1
90	1983	AWD	III A	Microscopic	Yes	Combination-2
89	1975	DOD	III A	Microscopic	Yes	Serous-1
89	1983	ANED	III C	Microscopic	Yes	Undifferentiated-3
75	1984	AWD	III C	Microscopic	Yes	Serous-3
68	1985	ANED	III A	Microscopic	Yes	Endometrioid-3
67	1985	ANED	III B	≤2 cm	Yes	Endometrioid-2

DOD, Died of disease; *AWD,* alive with disease; *ANED,* alive with no evidence of disease.

Table VII. Survival effect of cytoreduction of stages IIIB and IIIC disease (all patients receiving adjuvant chemotherapy)

Tumor stage	*Tumor residual*	*n*	*Survival rate*		
			5 year (%)	*Median (mo.)*	*p Value*
IIIA	Microscopic	8	47	50	
vs					NS
IIIB and IIIC	Microscopic	5	66	Not reached	
IIIB	≤2 cm	7	33	50	
vs					<0.005
IIIC	≤2 cm	13	0	25	

NS, Not significant.

the poorer the prognosis. Further, it was demonstrated that cytoreduction of stage IIIB and IIIC disease to no visible tumor mass (microscopic disease) resulted in long-term survival rates comparable with stage IIIA (Table VII). When stage IIIB and IIIC tumors could be cytoreduced to microscopic residual, the 5-year survival rates improved to 66% from the respective 29% and 5%. The three patients with stage IIIC disease who had tumors debulked to microscopic residual are still alive at 9 months, 75 months, and 89 months. Table VI demonstrates that the 5-year survivors all had either earlier stage IIIA or IIIB disease or had tumors that were cytoreduced at primary surgery to no visible residual tumor. Stage IIIC patients whose tumors were debulked to ≤2 cm were not able to obtain survival curves as good as stage IIIB patients left with ≤2 cm residuum (Table VII). Also, Fig. 5 reveals that the difference in stage IIIC patients between leaving bulky >2 cm residual disease or debulking to ≤2 cm is minimal and not statistically significant for improved long-term survival. Similar results have been reported indicating that tumor reduction to <2 cm may not be adequate.[10, 24] My study suggests that it is important to achieve as close to no visible tumor as possible at the completion of primary surgery to obtain significant long-term survival benefits. My study was not designed to determine the largest size beyond which benefit is lost. Griffiths suggests it should be at least <1.6 cm.[25] In a recent study, after stepwise combination of categories for an analysis of 268 patients, the best cutoff point for residual tumor size was found to be 1 cm.[26] Gynecologic Oncology Group studies now use the definition of <1 cm as "optimal" debulking.[27] Other studies have not found a magic cutoff that portends a better prognosis, but instead describe decreasing survival in gradual gradations of volume of residual disease.[28] It still seems to be unclear whether the ability to optimally debulk is more from the expertise of the surgeon or at least equally the effect of tumor biology and aggressiveness.[22] Potter et al.[28] found similar survival for patients who required bowel resection and radical techniques to obtain no gross residual disease compared with patients who even had gross residual disease remaining. However, Eisenkop et al.[29] reported at the 1990 annual meeting of the Society of Gynecological Oncologists that patients treated by gynecologic oncologists who debulked tumors to <1 cm residual in 76% of their cases versus 23% for other surgeons had higher survival rates at 3 years.

In spite of impressive initial response in many cases,[30] some recent articles have not demonstrated a clear long-term survival advantage of platinum-based combination chemotherapy over alkylating agents as initial adjuvant treatment of advanced ovarian cancer.[31] In my series survival was better for patients treated with platinum compared with alkylators only in the subgroup

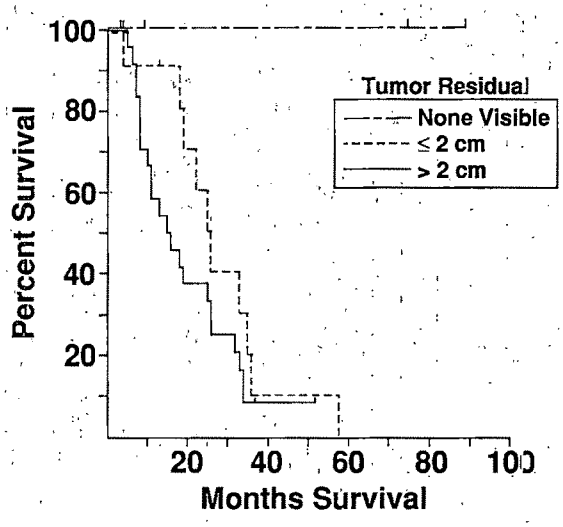


Fig. 5. Survival according to diameter of largest postoperative residual tumor in patients with stage IIIC disease treated with platinum-based combination chemotherapy. Chi-square test for trend yielded 6.2; $p \simeq 0.01$.

of stage IIIC patients with bulky remaining disease. Marsoni et al.[32] analyzed 852 stage III and IV patients from four trial regimens. Platinum-based chemotherapy improved survival rate when residual tumor was >2 cm but not in the subgroup with <2 cm residual tumor size.

Even with advanced adjuvant therapy, I could find no patient who survived to 5 years if there were >2 cm of residual disease after primary surgery. Other authors have reported long-term survivors (>60 months) who had >2 cm residuum. An analysis of 85 stage III and IV patients showed 8 of 20 long-term survivors had >2 cm residual disease after primary surgery.[10] Another noted an 8% 5-year survival rate for the patients who began adjuvant therapy with >2 cm residual disease.[33] As noted in Table VI, three patients are alive with no evidence of disease after 5-year survival. One study with a 10-year follow-up reported 18% 5-year, 11% 7-year, and 7% 10-year survival with no evidence of disease.[11] Another series with long-term follow-up had 18% of advanced ovarian carcinoma patients disease-free from 83 to 118 months after therapy.[12] Two other examples of no evidence of disease 5-year survivors include 13%[10] and 5 (8%) of 62 patients, with 4 having >2 cm disease at the time chemotherapy was initiated.[24]

There continues to be variation among studies as to the prognostic significance of other factors.[26, 34, 35] This study attempted to analyze prognostic factors by the stratification technique of the log-rank method. There was no significant correlation with survival found for age at primary surgery, presence of ascites, histology, grade, parity, speciality of primary surgeons, or prior hysterectomy.

The insidious nature of ovarian cancer results in late diagnosis. Inability to diagnose this disease early is shown by the 65% of epithelial ovarian carcinomas at these two hospitals that were stage III and IV a number that presumably would be even higher if the earlier stages I and II had more complete appropriate initial surgical staging. Moreover, most patients were initially seen with bulky upper abdominal disease; in this study stage IIIC represented 74 of the 101 cases. Symptoms were noted by the patients for an average of 1 to 4 months before the patient had exploratory laparatomy. Because the presenting symptoms were mostly abdominal and not pelvic in nature, 55 of the 101 patients were primarily operated on by nongynecologists. In only 28 cases did a gynecologist and general surgeon combine to complete the surgery. In this community over the period of my study only 23% of stage IIIC tumors were debulked to ≤2 cm diameter of residual tumor nodules. For the last half of my study the figure increased to 32%. Hacker[36] states that 85% of stage III patients' tumors should be debulked to ≤1.5 cm. It has been shown that primary cytoreductive surgery is safe[13, 37] and besides improving survival, does result in increased quality of life.[38] Progress in survival rate was demonstrated for the period 1980 to 1990 compared with 1970 through 1979. For stage IIIC patients this improvement most likely is caused by the increased number of patients whose tumors were cytoreduced (32% compared with 14%) and the value of platinum-based combination chemotherapy in suboptimally debulked tumors. This trend for improvement in survival rate is consistent with national statistics, which show that for all stages and histologic characteristics combined in white women the 5-year survival rate has increased from 36% for the period 1974 through 1976 to 39% during the years 1981 through 1986.[1]

There is no fully effective method of mass screening to detect ovarian cancer in its earlier stages.[39] It should be an achievable goal to diagnose these patients earlier and with more accuracy than is shown in this study. The primary care physician needs to consider the diagnosis in spite of the vague symptoms and quickly seek surgical consultation. Six patients serendipitiously diagnosed by a routine pelvic examination also suggest early diagnosis is beneficial. These asymptomatic patients possibly have improved survival, with only one dying so far; survival in the other five continues at 35 months to 89 months. If the protean symptoms of advanced ovarian cancer can be correctly identified as to source, referral to a gynecologic oncologist seems appropriate. However, if a patient has surgery in a community such as ours, safe tumor debulking may be accomplished by combining a gynecologist with a general-vascular surgeon who is competent operating in the deep vascular area of the pelvic retroperitoneum. This will require educating the medical staff that an aggressive surgical approach is indicated if minimal residual disease will be the result.

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Discussion

DR. GLEN E. HAYDEN, Seattle, Washington. Dr. Unzelman, your retrospective study of ovarian adenocarcinoma in two community hospitals involved a monumental task (review of 316 charts) that resulted in the selection of 101 FIGO stage III and IV cases.

As do other studies of ovarian carcinoma, your study shows that age, parity, the presence of ascites, the results of peritoneal cytology, and the tumor grade did not alter the prognosis. Your finding that 65% of the patients were in disease stage III and IV is in accord

with most studies, and your findings in regard to ultimate outcome closely match the results at major centers. The results of 1980 and 1990 show an improved prognosis with prolonged life expectancy. This study should result in better staging and initial debulking of advanced ovarian lesions at your hospital in the future. It is good that you point out the need for node sampling in stage III cases. A second factor in the improved survival rate was the recent addition of more effective chemotherapeutic agents, especially cisplatin and carboplatin. The immediate postoperative mortality rate of 5% is relatively high, but may reflect the older age of this group (median age 65 years).

This presentation clearly points out the need for early diagnosis. Family history cannot clearly identify the high-risk patient unless a first-order relative is involved, such as a mother or a sister. Yearly pelvic examination and pursuit of all adnexal masses are the foundation of good management. Additional tools now available are CA 125, ultrasonography, and laparoscopy. In their recent review of screening for ovarian cancer with CA 125 and ultrasonography Creasman and Disaia[1] agreed with the American College of Obstetrics and Gynecology committee opinion that these techniques are not suitable for routine screening.

The use of oral contraceptives definitely reduces the incidence of ovarian cancer by 50%. I believe that patients recognizable as being at high risk for ovarian cancer should be placed on oral contraceptives and continued on them regardless of age as long as they remain nonsmokers.

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DR. MICHAEL SMITH, Seattle, Washington. Two years ago I reported our experience with aggressive intestinal resection as part of the initial debulking of ovarian cancer.[1] At that time I was asked whether there was a survival advantage to such aggressive surgery. I was unable to answer the question, but I did feel that the quality of life was improved by reducing the need for subsequent colostomy or other intestinal surgery. I can now report that studies from Duke[2] and Alabama[3] have answered that question. Both reported no survival advantage. I fully agree with Dr. Unzelman concerning the need for complete primary debulking, but not to the extremes formerly recommended. I think that the data will also fail to show a survival advantage for patients who have undergone cavitron ultrasonic aspiration or argon-beam coagulation of difficult-to-resect diaphragmatic or mesenteric disease. Progress in this disease will come from advances both in early detection and in chemotherapy rather than from more aggressive surgery.

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DR. WILLIAM LUCAS, Ashland, Oregon. For the two and one-half decades before last year I was very involved with aggressive surgical attacks on ovarian cancer. During the last year I have had time to reflect on this surgical approach to ovarian cancer. I draw a different, and somewhat heretical, conclusion from the data Dr. Unzelman presented. It is far from proved that an aggressive surgical attack on ovarian cancer is always a valid approach to the treatment of this disease. It is primarily the intrinsic biologic characteristics of the tumor, including whether it is sensitive to chemotherapy, that determine the long-term prognosis. An aggressive initial attack is well validated. However, let me remind you that there are groups such as the West Midland Ovarian Cancer Group in England that have good data to show that an aggressive primary chemotherapeutic attack works almost as well, if not just as well, as an aggressive primary surgical attack, and that second-look surgery does not improve survival.[1] I am disturbed by aggressive secondary and tertiary surgery for ovarian cancer. Almost by definition, the patients subjected to secondary or tertiary debulking are those who have failed aggressive chemotherapy. Their tumors are not going to have a good response to surgery except in the very short term. In my view we are subjecting patients to a lot of morbidity, physical and psychologic, at great cost for questionable benefits.

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DR. ENRIQUE C.M. DECASTRO, Portland, Oregon. Dr. Hayden says that ultrasonography is too costly to be done on a routine basis; I am concerned because early detection of ovarian cancer should be our goal. Pelvic ultrasonography allows us to evaluate the pelvic cavity with greater certainty than bimanual examination alone.

Brachial plexus palsy: An old problem revisited

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Phoenix, Arizona

OBJECTIVES: It is an almost universal assumption in obstetric literature that brachial palsy is due to extreme lateral traction on the fetal head during the last phase of delivery. In contrast, there have been reports in the neurologic literature of probable intrauterine origin of brachial plexus palsy. Data to dispute or support the latter view were sought.
STUDY DESIGN: With this overview in mind, our perinatal data base was searched for all instances of shoulder dystocia and independently for all diagnoses of brachial plexus impairment.
RESULTS: Seventeen instances of brachial plexus impairment associated with shoulder dystocia were found. Twenty-two instances of brachial plexus impairment without mention of shoulder dystocia were ascertained. The characteristics of the two groups were remarkably different, especially in birth weight and in maternal age and parity.
CONCLUSIONS: The data are strongly suggestive that intrauterine maladaptation may play a role in brachial plexus impairment. Brachial plexus impairment should not be taken as prima facie evidence of birth process injury. (AM J OBSTET GYNECOL 1992;166:673-7.)

Key words: Brachial plexus impairment, shoulder dystocia, intrauterine maladaptation, medicolegal implications

Brachial plexus impairment appears to be a frequent and increasing reason for the instigation of medicolegal action. In a 5-year period in one hospital it was the reason for seven of 103 (6.8%) obstetrics-gynecology related lawsuits.[1] Indeed, some authors[2] stated that it was highly probable that in the future all cases of shoulder dystocia with a suboptimal outcome would be litigated. It is accepted by these and others that the injury always results from extreme lateral traction on the head during delivery of the shoulders. Since plaintiff's attorneys adduce evidence that the risk of shoulder dystocia is predictable and therefore brachial plexus damage is preventable, increasing attention is being given to this aspect of the problem.

On the other hand, little attention has been given to the possibility that brachial plexus impairments occur without evidence of birth trauma, just as do other nerve impairments such as facial palsy, isolated phrenic nerve impairment, and radial nerve palsy.[3-5] Furthermore, neurologic literature contains descriptions of brachial plexus impairments that were almost certainly of intrauterine onset.[6,7] Major evidence for intrauterine onset was based on electromyography. It takes approximately 10 days for muscle to develop electromyographic evidence of denervation; therefore such findings in the early neonatal period support an intrauterine onset of the impairment.

These observations led us to analyze our perinatal data base for data to either support or argue against the proposition that brachial plexus impairment may be caused by factors other than force occurring with delivery of the fetal shoulders.

Material and methods

The perinatal data system for our hospital has been previously described.[8] Data for 57,597 births occurring from 1977 through 1990 were analyzed for distribution by maternal age and parity, delivery mode, gestational age and birth weight, Apgar scores, and need for admission to the intensive care nursery. Separate searches for all recorded diagnoses of shoulder dystocia and brachial plexus impairments were conducted. If other peripheral nerve damage had occurred in conjunction with brachial plexus impairment, these were ascertained, but no separate search for other peripheral nerve damage or skeletal trauma was carried out.

Results

Thirty-nine infants were diagnosed as having incurred brachial plexus impairment. The impairment ranged from weakness of an upper extremity in three infants to a specific diagnosis of brachial (Erb's) palsy in the other 36. No diagnoses of lower plexus (Klumpke's) palsy were recorded. Seventeen of the brachial plexus impairments were associated with a recorded diagnosis of shoulder dystocia whereas 22 had

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Table I. Brachial plexus impairment with and without mention of shoulder dystocia

Maternal characteristic	With specific diagnosis		Without specific diagnosis	
	No.	%	No.	%
Maternal age <20 yr	1/17	6	7/22	32
Nulliparous	5/17	29.4	14/22	64
Multiparous	12/17	70.6	8/22	36

Table II. Brachial plexus impairment with and without mention of shoulder dystocia

Labor or delivery characteristic	With specific diagnosis		Without specific diagnosis	
	No.	%	No.	%
Delivery mode				
Spontaneous	4/17	24	9/22	41
Low-forceps or vacuum	12/17	71	7/22	32
Midforceps	1/17	6	3/22	13
Breech vaginal	0/17	0	2/22	9
Cesarean section	0/17	0	1/22	4
Second stage >1 hr	9/17	53	7/22	32

no such association. The contrasting characteristics of the two groups were analyzed and are presented in a series of tables. In the appropriate tables "with specific diagnosis" indicates the group for which this diagnosis was recorded and "without specific diagnosis" indicates that this diagnosis was not recorded for this group.

The contrasting maternal characteristics are shown in Table I. Approximately a third of the mothers in the group with no recorded shoulder dystocia were <20 years old whereas only one of the 17 with shoulder dystocia was in this age group ($\chi^2 = 4.496$, $df = 1$, $p < 0.05$). The contrast in parity was even sharper. Sixty-four percent of the non-shoulder-dystocia group were primigravid compared with 29% of the shoulder-dystocia group ($\chi^2 = 3.956$, $df = 1$, $p < 0.05$).

Labor and delivery variations are listed in Table II. Spontaneous vaginal delivery was more than twice as common in the non-shoulder-dystocia group with slightly more than half of these infants being delivered in that manner. These differences, although clinically impressive, were not statistically significant ($\chi^2 = 1.303$, $df = 1$). Vaginal breech delivery accounted for two of the brachial palsies in the non-shoulder-dystocia group. One infant at 30 weeks' gestational age was delivered as a frank breech and weighed 1786 gm. The other, a compound breech, was delivered at 34 weeks and weighed 2934 gm. One infant in this group was delivered by cesarean section without mention of any delivery problem, including delivery of the shoulder. The 41-week infant weighed 2572 gm. The infant was in a transverse lie presentation with the umbilical cord around the neck two times. Although a second stage >1 hour was only slightly more common in the shoulder-dystocia group, this was probably made more significant by the fact that only 29% of nulliparous women were in this group as compared with 24% in the non-shoulder-dystocia group.

Table III. Brachial plexus impairment with and without mention of shoulder dystocia

Infant characteristic	With specific diagnosis		Without specific diagnosis	
	No.	%	No.	%
Gestational age				
<36 wk	1/17*	6	5/22	23
>40 wk	7/17	41	3/22	14
Birth weight				
1501-2500 gm	0/17	0	3/22	14
2501-3500 gm	1/17	6	10/22	46
3501-4000 gm	4/17	24	4/22	18
4001-4500 gm	7/17	41	2/22	9
>4500 gm	5/17	29	3/22	13

*Infant of diabetic mother born at 34 weeks and weighing 4253 gm.

Contrasts in infant characteristics are shown in Table III. Five of the 22 infants in the non-shoulder-dystocia group were <36 weeks as compared with only one in the shoulder-dystocia group ($\chi^2 = 2.090$, $df = 1$, NS). The only preterm infant in the shoulder-dystocia group was born to a diabetic mother at 34 weeks and weighed 4253 gm. The five preterm infants in the non-shoulder-dystocia group were born at 30, 31, 33, 34, and 34 weeks. On the other hand, 41% of the shoulder-dystocia group were >40 weeks' gestation as compared with only 14% of the non-shoulder-dystocia group ($\chi^2 = 3.815$, $df = 1$, $0.10 > p > 0.05$).

Contrasts in birth weight were more impressive (Table III and Fig. 1). Thirteen of the 22 infants (60%) of the non-shoulder-dystocia group weighed <3500 gm as compared with only one in the other group. Twelve of 17 (70%) in the shoulder-dystocia group weighed >4000 gm as compared with 5 of 22 (44%) is the non-shoulder-dystocia group ($\chi^2 = 13.138$, $df = 3$, $p < 0.01$). It is of interest that the largest baby, who weighed 5727 gm and was delivered spontaneously, was not recorded as having shoulder dystocia.

Certain aspects of birth condition are listed in Table IV. Sixty-five percent of the shoulder-dystocia group had a 1-minute score of <7 compared with 36% of the non-shoulder-dystocia group ($\chi^2 = 3.083$ $df = 1$, $0.10 > p > 0.05$). At 5 minutes 41% of the shoulder-

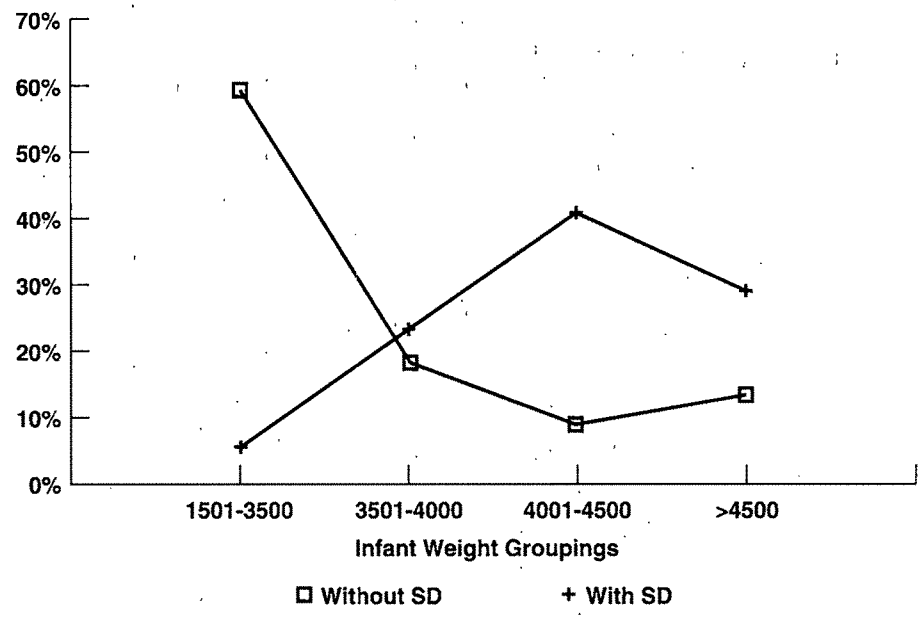


Fig. 1. Weight groupings by percentage of infants with brachial palsy with and without associated diagnosis of shoulder dystocia *(SD)*.

dystocia group had a score of <8 compared with 14% of the non-shoulder-dystocia group ($\chi^2 = 3.815$, $df = 1, 0.10 > p > 0.05$). These differences are suggestive but not statistically significant.

The only associated nerve involvements occurred in the non-shoulder-dystocia group. No clavicular fractures were recorded in either group. However, one infant in the non-shoulder-dystocia group who was delivered spontaneously after a 36-minute second stage had bilateral femoral fractures, as well as bilateral brachial plexus palsy. Gestational age was 37 weeks and birth weight was 2586 gm.

The question of possible underrecording of shoulder dystocia is a legitimate one, and the incidence of shoulder dystocia with associated brachial plexus impairment by year and by weight groupings was analyzed and is displayed in Table V.

Table IV. Brachial plexus impairment with and without mention of shoulder dystocia

	With specific diagnosis		*Without specific diagnosis*	
Condition at birth	*No.*	*%*	*No.*	*%*
Apgar score				
<7 at 1 min	11/17	65	8/22	36
<8 at 5 min	7/17	41	3/22	14
Neonatal intensive care unit admission	7/17	41	10/22	45
Other nerve impairment				
Phrenic nerve	0/17	0	1/22	5
Facial palsy	0/17	0	1/22	5
Bilateral brachial palsy	1/17	6	3/22	14

Comment

As reflected in the title of this article the focus is on brachial plexus impairment and only secondarily on shoulder dystocia. The term *brachial plexus impairment* rather than *brachial plexus injury* was used advisedly, inasmuch as the latter term carries with it the connotation that direct physical trauma is always involved.

In this large group of patients whose infants were delivered over a 13-year period, 22 of 39 (56%) cases of brachial plexus impairment were not associated with recognition of difficulty with delivery. Indeed, one was associated with cesarean section. Gonik et al.[9] made the same observation in a smaller group of patients in which 71% of infants with shoulder girdle impairment did not have shoulder dystocia recognized in the delivery process. Those authors attributed this lack of recognition to more subtle instances of shoulder dystocia without clinician recognition of the problem.

We concede that a lack of recognition of shoulder delivery problems may be involved in some of the brachial plexus impairments in this group. However, the contrasting characteristics strongly suggest that other factors are involved. The differences in maternal age and parity are significant. If the impairments were associated only with subtle, unrecognized shoulder dystocia, it would be expected that age and parity would be similar in the two groups. Actually, maternal age <20 was five times greater in the non-shoulder-dystocia group and nulliparity was more than twice as common.

Table V. Shoulder dystocia and associated brachial plexus impairment by year

Year	No. of births	Shoulder dystocia		Brachial plexus impairment	
		No.	%	No.	%*
1977	3,071	25	0.8	1	4
1978	3,193	13	0.4	1	7.7
1979	3,649	19	0.5	0	0
1980	4,028	23	0.6	1	4.3
1981	4,102	22	0.5	3	13.6
1982	3,577	21	0.6	3	14.3
1983	4,255	28	0.7	2	7.1
1984	4,452	37	0.8	0	0
1985	4,531	40	0.9	0	0
1986	4,359	46	1.1	3	6.5
1987	4,486	32	0.7	0	0
1988	4,837	48	1	0	0
1989	4,413	62	1.4	1	1.6
1990	4,644	56	1.2	2	3.6
TOTAL	57,597	472	0.8	17	3.6

*Percent of infants with shoulder dystocia and brachial plexus impairment.

It has been postulated that intrauterine pressures associated with uterine anomalies could be a factor[5] Although the presence of such anomalies could not be determined from the available data, uterine maladaptation associated with young maternal age and nulliparity might well be associated with a higher incidence of intrauterine pressures resulting in nerve impairment. Spontaneous vaginal delivery was more than twice as common in the non-shoulder-dystocia group, suggesting less probability of difficulty in delivery. Certainly, the brachial plexus impairment in a 2572 gm infant delivered by cesarean section from a transverse lie would have a high probability of intrauterine maladaptation as a cause.

The increasing incidence of diagnosed shoulder dystocia during the period of this study without an increase in associated brachial plexus impairment deserves comment. Rather than an underrecording of shoulder dystocia, it would speak to an increasing awareness of the medicolegal implications of the condition and a tendency to record even minor degrees of difficulty. This in turn would make subtle, unrecognized shoulder dystocia a less likely cause of brachial plexus impairment.

Weight distribution is another significant factor. Almost 60% of the infants in the non-shoulder-dystocia group weighed <3500 gm with approximately one third weighing <3000 gm. The probability of unrecognized problems in this weight group is low. On the other hand, the possibility of a uterine maladaptation problem could exist as well with the larger infant. The occurrence of brachial plexus impairment in five preterm infants is inconsistent with shoulder dystocia inasmuch as shoulder circumference is almost always less than head circumference.

The occurrence of more instances of brachial plexus impairment without recognizable problems with the delivery of the shoulder than when such a problem is perceived renders basically untenable an argument that brachial plexus and other related nerve impairments are predictable. Nor can brachial plexus impairment with diagnosed shoulder dystocia be taken as prima facie evidence that the two are causally related in all such instances.

The role of electromyography in identifying possible onset of brachial plexus palsy during the antenatal period as suggested by Koenigsberger[6] was not addressed during this study but will be the focus of an anticipated subsequent report.

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Discussion

DR. CARL GOETSCH, Berkeley, California. Dr. Jennett and colleagues have made a significant contribution to the growing evidence that not all untoward outcomes of delivery are caused by events during the process of labor and delivery. They have shown that such outcomes may, in fact, result from conditions that antedate delivery or even labor. This article clearly shows that although impaired function of the brachial plexus may have been associated with shoulder dystocia (46%), an even larger group (54%) had no dystocia. In fact, one case was delivered by cesarean section. Dr. Jennett listed findings that differentiate the two groups and cited the reports from as early as 1956 by pediatricians and neurologists who pioneered in studies of this problem.

In spite of its limits, this report is important because it enlarges the literature on the problems of development and function encountered in both the gestational and parturitional phases of our arrival on this troubled planet. A partial solution to one problem that plagues

the obstetrician, the threat of professional liability, is expansion of our knowledge of the causes of these dysfunctions. With this knowledge, the expert witness increases his competence, the defense attorney is armed, and the plaintiff's attorney is, to the same degree, disarmed.

Dr. Jennett, in his discussion, points out the desirability of using the term *brachial plexus impairment* instead of the term *injury*, which offers the opposing attorney the opportunity to argue that the physician caused the injury. I add as alternatives *dysfunction* or the old term *palsy*. Good defensive record keeping requires cultivating our sensitivity to the semantic implications of the words we use.

Dr. Jennett promises to continue to study other aspects of brachial plexus palsy, especially the use of electromyography as a diagnostic tool. Considering the low incidence of brachial plexus palsy, that is a formidable task. I congratulate Dr. Jennett and his team for their imaginative extraction of the "gold" sequestered in their computer data bank in Phoenix.

Dr. Julian T. Parer, San Francisco, California. It is my impression that many plaintiff's lawyers are reluctant to press shoulder dystocia cases because it is clear that prediction of shoulder dystocia is extremely difficult. It is also difficult to show that the physician delivering the infant did not go through all the appropriate steps. It is interesting to read depositions by the defendant obstetricians because they almost cite chapter and verse out of *Williams' Obstetrics* in describing exactly what they did.

With regard to the method of delivery after shoulder dystocia is recognized, I was taught to avoid putting heavy traction on the anterior shoulder and, if other appropriate steps do not work, to deliver the posterior arm by the strenuous technique of forcing my hand up posteriorly to grasp the forearm. Can you tell us about the method of delivery used for the infants who had shoulder dystocia?

Dr. Lester T. Hibbard, Carmel, California. Did all these infants have a permanent injury or did some have a temporary injury?

Dr. James D. Fearl, Portland, Oregon. In how many of these cases of shoulder dystocia was the impairment identified by the obstetrician after delivery? I think that today there is a tendency for obstetricians not to carefully evaluate healthy-appearing babies. You will have better rapport with your patient if you yourself identify the impairment, rather than being called later by the nursery or, even worse, being told by the mother when you return on postpartum rounds.

Dr. Philip E. Young, San Diego, California. I disagree with Dr. Parer and believe that shoulder dystocia malpractice cases will be increasingly common. I have appeared as an expert witness for the defense in five cases of shoulder dystocia and brachial plexus palsy. The defense prevailed in each. In one, the judge threw the verdict for the defense, citing from *Williams' Obstetrics* the comment to the effect that care must be taken not to apply excess lateral traction because such application may cause a brachial plexus injury. This statement was interpreted by the judge to mean that this is *the* way the injury occurs in cases of shoulder dystocia, not just *a* way.

Dr. Jennett pointed out that at least half of these brachial plexus impairments may not be related to shoulder dystocia at all. I would say further that even when the impairment is associated with shoulder dystocia, it may be quite independent of any lateral traction applied by the obstetrician. I was once present at a delivery in the emergency room. My partner, having been called in to see a very large lady with no prenatal care who was just about to deliver, called me in because he thought he might encounter shoulder dystocia. The patient was pushing, but neither of us was prepared to do anything that instant because we were still gowning. Out came the head and immediately it retracted back. Every medical person in the room recognized this as an obvious case of shoulder dystocia. Then the woman yelled and pushed again. The baby went sailing and was just barely caught by my partner. The infant, untouched by human hands until after it was delivered, had brachial plexus palsy. I believe that the mechanism of injury in this case was the shoulder dystocia itself. The baby had a large stubby neck and the mother's obesity made her birth canal effectively much longer. For some patients shoulder dystocia and brachial palsy are indeed associated, but the association is not that the obstetrician placed traction on the head. The association is that the forces of labor and delivery place traction on the plexus.

If the members of this society who write obstetrics textbooks would see that this information provided by Dr. Jennett is promulgated, it would be a big help to practicing obstetricians.

Dr. Jennett (Closing). Dr. Parer, the methods used to deal with shoulder dystocia are not recorded in the computerized record. An analysis of that question would require a search of each microfiche record, and we did not do this because brachial plexus impairment was our focus. We did determine whether the delivery was done by a resident or by a member of the attending staff. Twenty-five percent were done by the residents and 75% were done by the attending staff. In our hospital 25% of the patients are cared for by the residents and 75% by the attending staff. This led us to believe that in this situation experience does not have much to do with the outcome.

Dr. Hibbard, the impairment was temporary. Dr. Tarby, our pediatric neurologist who examined most of the infants with brachial plexus impairment born during the last 7 years, does not recall any who had a permanent impairment.

Dr. Fearl, I think that often the obstetrician did not known that the infant had incurred a brachial plexus palsy. The obstetrician who delivered one baby who had bilateral brachial plexus palsy recently told me that, although it was an important subject, he himself had never delivered a baby with brachial palsy.

Modified retropubic cystourethropexy II

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In 1986 we published a preliminary report describing a modification for bladder-neck suspension in the treatment of urinary stress incontinence. From 1982 through 1989 135 surgical procedures were performed. Long-term follow-up of these patients is now presented. Of the 135 patients in this study, 118 were available for follow-up at the time of this report; 108 (91.5%) of the 118 reported no urinary leakage. Of the 10 patients who reported continued urinary leakage, only 2 had persistent or recurrent urinary stress incontinence; they were reoperated on with favorable results. The other eight patients reporting urinary leakage were patients with mixed-cause urgency incontinence. The adjusted success rate for the treatment of genuine stress incontinence was therefore 98%. (Am J Obstet Gynecol 1992;166:1678-82.)

Key words: Urinary stress incontinence, urgency incontinence, modified retropubic cystourethropexy

As more women look to the gynecologist as their primary care physician, the gynecologist is confronted with an increasing number of women who have urinary incontinence. It is anticipated that as women live longer into the postmenopausal years the incidence of urinary incontinence will increase. Brockelhurst et al.[2] found that 57% of women ages 45 to 64 years in a general practice population had stress incontinence. Norton[3] pointed out that some patients assume that the problem is normal and do not seek medical advice; therefore the physician must carefully question patients about their urinary function.

The literature is replete with articles on the subject of urinary stress incontinence, with over 100 procedures described.[4] Physicians must choose the appropriate surgery for each individual on the basis of careful study of the patient's pathophysiologic findings.

Our original publication reviewed the short-term results of 26 patients who underwent modified retropubic cystourethropexy between December 1982 and April 1985.

Our present study is an ongoing analysis of this procedure, which was further modified in 1987 by substituting cystotomy with suprapubic telescopy.

Material and methods

Operative technique. All patients were given a preoperative antibiotic, usually a second-generation cephalosporin, intravenously just before surgery. At the time of surgery the pelvic examination was repeated with the patient under anesthesia, and urethral dilatation was performed if indicated. The patient was placed in the "frog-leg" position and the abdomen, vagina, vulva, and inner thigh areas were prepared and draped in a sterile manner. A transurethral Foley catheter was inserted into the bladder and its balloon inflated with 5 to 10 ml of water. A Y connector was attached to the catheter so that both bladder drainage and filling could be accomplished as required.

The procedure was usually performed through a transverse suprapubic incision. In obese patients or patients in whom added exposure was necessary, a Maylard muscle-splitting incision was used. Hysterectomy or other indicated intraperitoneal procedures were performed, and the peritoneum closed before the retropubic space was entered. The paraurethral fatty tissue was carefully dissected away, and a Babcock clamp was placed around the urethra just below the ureterovesical junction. With two fingers in the vagina as a guide, a No. 1 Novafil suture was placed in the vesicovaginal fascia on each side of the bladder neck approximately at right angles to the urethra, with a helical technique (Fig. 1).

Indigo carmine dye was given intravenously, and the bladder was retrofilled with 400 ml of water. A size 00 chromic purse-string suture was placed in the dome of the bladder, and a needle-tip cautery was used to create a small cystotomy within the sutured area. The telescope of the cystoscope (without its sheath) was inserted into the bladder through this cystotomy, and the bladder was inspected for inadvertent suture penetration. The ureteral orifices were visualized until blue-dyed urine was spurting from each side. The telescope was withdrawn, and a No. 14 silicone elastomere Foley catheter placed through the same cystotomy incision; the catheter balloon was inflated and the purse-string su-

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ture securely tied around it. The catheter exited the abdomen through a stab incision just inferior to the suprapubic incision in the right lower quadrant. The retropubic space was drained with a No. 10 Jackson-Pratt suction drain (Heyer-Schulte, Irvine, Calif.) exiting through a stab incision in the left lower quadrant of the abdominal wall.

The swaged needles of the Novafil suspensory sutures were cut, and the two ends of one suture were threaded onto a free Mayo needle; the needle was passed through the inferior aspect of the muscle and fascia just above the symphysis pubis 1 to 2 cm lateral to the midline. The same procedure was repeated on the opposite side.

After closure of the rectus fascia the two suspensory sutures were tied to each other across the inferior aspect of the rectus fascia so as to elevate the bladder neck. The degree of elevation of the bladder neck was determined with a small urethral dilator or cotton-tip applicator placed into the urethra; the urethral axis was estimated while the suspensory sutures were tied down (Fig. 2). A urethral axis of 10 to 20 degrees below the horizontal was considered optimal for this suspension procedure.

Postoperatively, the suprapubic catheter was connected to gravity drainage for the first 24 hours. On the second postoperative day the catheter was plugged, and the patient was allowed to void or to attempt to void. The residual urine was measured after each voiding. When voiding was adequate (that is, a urine residual consistently <100 ml) the catheter was removed. Patients were usually discharged on the third or fourth postoperative day with or without the suprapubic catheter. If they were not yet voiding adequately, they were instructed to call the office when they were able to void without significant residuals. Those patients discharged with their suprapubic catheters were given prophylactic antibiotics, such as Bactrim DS. More recently, in selected patients in whom only cystourethropexy was performed the procedure has been done on an outpatient basis and the patient has been discharged the same day.

Evaluation and patient characteristics. The procedure was performed on 135 patients between December 1982 and December 1989. The two authors and an associate performed all of the surgeries. Patient evaluation before surgery was detailed in our previous publication.[1] We usually used a simplified urogynecologic work-up as described in a recent publication of the American Uro-Gynecological Society.[5]

Forty-seven (34.8%) patients had preoperative urethrocystoscopy and cystometry. Two patients had multichannel urodynamic assessments. Patients who had urinary stress incontinence but who exhibited genital prolapse were operated on transvaginally; indicated surgery was performed together with a modified Per-

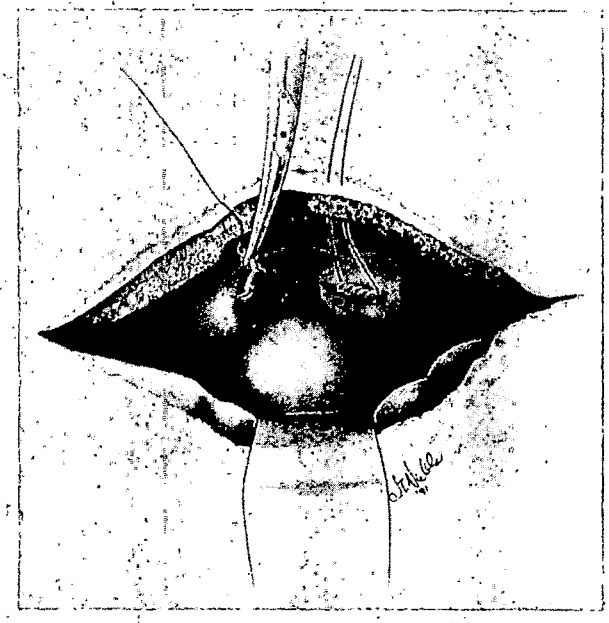


Fig. 1. Helical suture in paraurethral fascia.

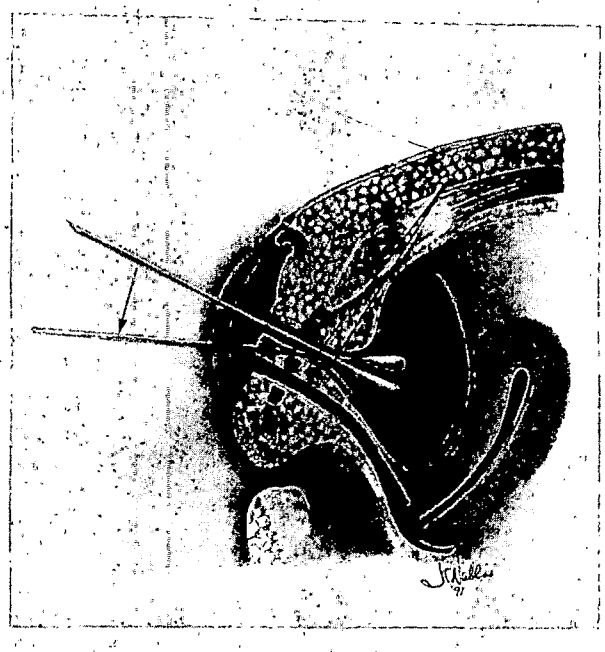


Fig. 2. Cotton-tip applicator in urethra as guide to change in urethral axis with tying down of suspensory suture.

eyra suspension of the vesical neck. Our article deals only with those patients who had a transabdominal retropubic procedure.

Forty (29.6%) patients underwent modified retropubic cystourethropexy only; 95 (70.3%) had additional surgery such as hysterectomy or salpingooophorectomy. Patients ranged in age from 27 to 72 years; 47 (34.8%) patients were >50 years of age. Postoperative follow-up time for the patients in this study was from

Table I. Overall results

135 Surgeries (1982-1989)
118 Patients available for follow-up
108 Patients dry (108/118) (91.5%)

Table II. Analysis of failures

10 Patients with persistent incontinence
8 Mixed-cause incontinence with persistent urge incontinence
2 Recurrent or persistent stress incontinence

1 to 8 years. Seventy (51.8%) patients have been monitored for ≥4 years since their surgery.

Results were assessed by physical examination and each patient's subjective report of her urinary status. Objective testing, including urodynamic studies, was not routinely performed postoperatively in this clinical study.

Results

All charts were reviewed and patients were contacted by telephone in January 1991. Two patients had died, and 15 had either moved from the area or were lost to follow-up. One hundred eighteen patients were available for follow-up. One hundred eight (91.5%) patients reported that they did not experience any urinary leakage (Table I). Ten patients continued to have urinary leakage after surgery (Table II). Eight of these patients had persistent urgency incontinence. All but one of these eight patients had preoperative urethrocystoscopy and cystometric studies and were diagnosed as having mixed-cause incontinence (i.e., anatomic incontinence and detrusor instability) preoperatively.

Of the two patients whose surgeries were classified as failures to correct anatomic stress incontinence, one patient noted recurrent stress incontinence 2 years postoperatively and was reoperated on by us in 1987. At her interview in January 1991 she reported that she was dry. The other patient who did not experience relief in her urinary incontinence had received pelvic radiation for adenocarcinoma of the endometrium 2 years before her retropubic cystourethropexy. In addition, she had been classified as having mixed-cause incontinence after her preoperative urethrocystoscopy and cystometric study.

Of interest is the fact that 2 years after an unsuccessful outcome with our procedure, a Raz procedure[6] was performed; the patient reports considerable improvement, although she is not completely dry. Excluding the eight patients with mixed-cause incontinence who continued to experience urgency incontinence, the adjusted success rate in anatomic urinary stress incontinence for this procedure was 98.3%.

Comment

The basic principles in our surgical modification are (1) the use of a helical, nonabsorbable suture in the paraurethral fascia, (2) a suprapubic cystoscopy to rule out inadvertant urinary injury, (3) the use of a cotton-tip applicator or urethral dilator in the urethra to gauge the urethral axis and prevent possible overcorrection, and (4) the fixation of the suspensory suture to the anterior rectus fascia just above the symphysis pubis.

The advantage of the helical suture is that it produces a thickening and pleating of the paraurethral tissues to prevent the suspensory suture from pulling out of the tissue; this advantage has been described by Pereyra and Lebherz.[7]

With the use of permanent suture the possibility of inadvertent bladder penetration must be considered; when performing suprapubic cystoscopy we have observed on occasion a pass of the Novafil suture in the bladder and have simply removed and repositioned the suture without any subsequent problem. We have observed no fistulas or permanent urinary injuries in any patients.

With any type of cystourethropexy to correct anatomic urinary stress incontinence, the possibility of overcorrection of the urethral axis exists. Overcorrection may result in obstruction with large postvoid residuals and inefficient voiding with bladder instability, frequency, and urgency.[8] By using an object in the urethra to gauge the urethral axis while tying down the suspensory suture, there is less chance of overcorrection. We have occasionally seen transient high postoperative urinary residuals requiring urethral dilations, but no patients have had permanent voiding dysfunction or required takedown of the repair.

The use of the anterior rectus fascia to anchor the suspensory suture proved quite effective; in the one failure that was reoperated on by us the suspensory suture had pulled out of the paraurethral fascia, but the suture in the rectus fascia was intact.

Although the modified retropubic cystourethropexy is more time-consuming than the needle-suspension procedures,[9] results appear to be superior to these procedures and appear to be comparable to the Burch procedure.[10]

The follow-up time in this study (1 to 8 years) is of relatively short duration to provide objective criteria for judging this procedure's effectiveness; as is pointed out by Benson et al.,[11] a 5- to 10-year follow-up period is required to properly evaluate any antiincontinence procedure. Further underscoring the need for long-term follow-up, Stanton[12] reported a 94% cure rate for the Burch procedure at 1 postoperative year and 77% at 5 postoperative years.

The failure of this procedure to correct the urgency component of incontinence in the 8 patients with

mixed-cause incontinence points up the need to carefully review and discuss the possibility that surgery may not result in these patients being totally dry postoperatively.

Whether antiincontinence surgeries should be performed in patients with mixed-cause incontinence has been controversial. Stanton[12] has suggested that the degree of rise in detrusor pressure on cystometric evaluation of those patients with detrusor instability may be an important predictive observation of potential failure. According to Kohorn,[13] in patients with mixed-cause incontinence, the less severe the anatomic defect the more likely it is that the associated detrusor instability will subside after bladder-neck suspension surgeries.

An excellent study by Ramon et al.[14] concluded that a good anatomic repair was the most important factor in the management of incontinent patients who exhibit hypermobility of the bladder neck regardless of the coexistence of urethral weakness or detrusor instability.

As we continue to use our modification of the retropubic cystourethropexy we are encouraged by its relative simplicity and lack of postoperative voiding problems. The 98% clinical cure rate for genuine stress incontinence over the 1- to 8-year observation period in this series is encouraging, and we feel that this procedure belongs in the repertoire of the urogynecologist.

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Editor's note: This manuscript was revised after the discussions were presented.

Discussion

Dr. David Pent, Paradise Valley, Arizona. This is a welcome follow-up report on a surgical technique that Dr. Goodno first reported to this Society 6 years ago. At that time his series consisted of 26 patients monitored for 10 months to 4 years. Now he presents five times as many patients with a follow-up period of 1 to 7 years, with the majority having been operated on ≥4 years ago.

Regarding Dr. Goodno's reported cure rate, these figures are based on information reported by the patient in a telephone interview. Because the International Continence Society definition of incontinence involves it being a social and hygeinic problem, it seems appropriate to consider the patient cured on the basis of her statement that her incontinence is no longer a problem. However, some patients' replies might be influenced by wanting to please the doctor or by fear of having to undergo a repeat surgery, so an objective measurement of incontinence, such as a perineal pad–weighing test, would seem to be of value and would help in comparing these results with those of other authors.

Most of Dr. Goodno's surgical failures involved patients who exhibited mixed incontinence—both genuine stress incontinence and detrusor instability—on preoperative testing. I wonder whether these patients were tested postoperatively to confirm that their persistent incontinence was caused by the detrusor instability, because symptoms alone can be very misleading. I am a little disturbed over dropping these uncured mixed-cause incontinence patients from the calculation of the stress incontinence success rate because those patients with mixed incontinence who were cured were counted. I think it would be better to treat these as two separate groups and report a success rate for each. This would also help shed some light on the role of surgery in the presence of mixed incontinence which, as Dr. Goodno notes, is controversial to say the least. In any event his success rate of 108 patients in the 118 on whom follow-up could be obtained is excellent and is comparable to the Burch procedure.

Dr. Goodno notes that many women with incontinence do not seek medical advice, and I believe that there are two main reasons. First, as he notes in quoting

Norton, many women feel that this is a normal part of aging. Indeed, this is an essential part of the individual's coping mechanism—it is easier to cope with a "normal" process than to deal with an illness. And the fact that June Allyson talks about Depend undergarments on television and that supermarket shelves are stocked with protection apparel only serves to reinforce the idea that incontinence with aging is normal.

Second, many patients are afraid to discuss their incontinence problems because they assume surgery will be necessary. We need to make women aware that nonsurgical modalities can help. The use of biofeedback, physiotherapy, weighted vaginal cones, and electric stimulation have enhanced the effectiveness of pelvic-floor–muscle exercises and have demonstrated a high degree of success. In the past many gynecologists have been disappointed in the results of the Kegel exercises, but numerous studies have shown that most patients who believed they were doing Kegel exercises were really doing nothing more than contracting abdominal and gluteal muscles and not their perineal musculature.

Dr. Goodno's cystourethropexy procedure is relatively simple to perform; his excellent cure rate, along with the lack of postoperative voiding problems, recommends this technique to any surgeon dealing with urogynecology. As he has noted, long-term follow-up is essential, and I look forward to hearing a further update of the results in another 5 to 6 years.

DR. HOWARD BLANCHETT, Berkeley, California. Do you think that as we approach the 21st century there will be a declining incidence of stress incontinence because of declining parity, declining operative vaginal deliveries, and increasing emphasis on women's physical fitness?

DR. GOODNO (Closing). Dr. Pent, you brought up the important issue of the nonsurgical management of the incontinent patient, including pubococcygeus muscle exercises and biofeedback. We heartily agree that some patients may be able to avoid surgical repair by diligently adhering to exercise program. Additionally, you correctly observed that ours was a study with a subjective endpoint, the patient's statement that she was continent or incontinent, which was not verified by urodynamic studies. I agree that we might have made important observations had we reported success rates after segregating patients with mixed-cause incontinence from those with pure anatomic incontinence.

Dr. Blanchette, I do not know whether we can anticipate a decline in the incidence of urinary stress incontinence because today's women have fewer pregnancies, receive better obstetric care, exercise regularly, and are generally more concerned with their health. However, because of increased life expectancy, we doctors will still have many patients with incontinence in our care.

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The physiologic mechanisms of variable decelerations

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OBJECTIVES: The purpose of this study was to determine the cause and physiologic consequences of variable decelerations.
STUDY DESIGN: Previous studies of heart rate changes in human and experimental animals were critically reviewed with respect to the autonomic nervous system component, the cause of the increased vagal activity, and the role of cord compression or other stimuli in these heart rate changes.
RESULTS: There is abundant evidence from experimental and human studies that variable decelerations can be reproduced by either cord compression or head compression. The vagal reflex produced is probably caused by a combination of chemoreflex (earlier in the deceleration) and baroreflex (later). The variable deceleration is accompanied by an acidosis, primarily respiratory, and probably hypoxemia. Cord compression results in decreased umbilical blood flow.
CONCLUSIONS: Recent Doppler velocimetry studies suggest that even though the variable decelerations may be similar in duration and depth, the reduction of umbilical blood flow may be greater when the prime cause is cord compression than when the prime cause is vagal reflex from another source. (AM J OBSTET GYNECOL 1992;166:1683-9.)

Key words: Variable decelerations, cord occlusion, head compression, Doppler ultrasonography

Variable decelerations of the fetal heart rate (FHR) are characterized by their abrupt onset and recovery, variable shape, and variable relationship to contractions.[1] In contrast to late decelerations they are considered less ominous because their association with fetal acidosis appears to be weaker.[2] There is general agreement that variable decelerations are produced by a vagal response of the fetus. However, the stimulus for the vagal activity is less certain. In this report we will examine the evidence for the mechanisms of variable decelerations and discuss the potential importance of different mechanisms in the management of the fetus during labor.

Umbilical cord occlusion

Barcroft[3] in 1947 occluded the umbilical cord of the fetal goat, producing a deceleration of a type now considered as variable deceleration, which was abolished by vagotomy. Since then numerous investigators have performed studies involving occlusion of the umbilical cord or its component vessels in both experimental animals and humans. The studies have been carried out on either anesthetized or chronically prepared animals, the occlusion has been partial or complete, and the duration of occlusion has varied from several seconds up to 1 hour. In a number of the studies fetal blood pressures and arterial blood gases have also been measured. In addition, the involvement of the autonomic nervous system has been examined.

Heart rate changes during umbilical cord occlusion. As with the work of Barcroft, the majority of workers have reported an abrupt decrease in heart rate with cord occlusion. Studies have been carried out in fetal sheep,[4-9] goats,[10] baboons,[11] and humans.[12, 13] The duration of occlusion has ranged from 5 seconds to >60 minutes, and either umbilical arteries alone, umbilical veins alone, or the whole of the umbilical cord has been occluded. In some cases the occlusion is described as partial and in other cases as complete.

The responses to umbilical arterial occlusion are similar, when reported, to those of umbilical cord occlusion of all vessels. The initial response is a decrease in heart rate, although some workers under some conditions have reported a tendency for fetal heart rate to return toward normal values. Thus Towell and Salvador[10] noted that there was a bradycardia for the first 5 minutes of occlusion, but this reverted toward normal in the second 5 minutes of occlusion. Ball et al.[9] noted that the initial bradycardia returned toward a normal heart rate approximately 10 minutes after severe umbilical arterial occlusion producing an oxygen satura-

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tion below about 10% in the fetal arterial blood. Goodlin and Lowe[13] noted a deceleration with subsequent acceleration with 1 to 2 minutes of occlusion in the premature human fetus.

With respect to umbilical vein occlusion alone, most workers similarly report an abrupt drop in heart rate. However, there are some exceptions, and James et al.[11] in the anesthetized (fluothane) fetal baboon noted that with partial mild occlusion of the umbilical vein that there was a tachycardia, although with complete occlusion there was a bradycardia. Their occlusion was from 0.2 to 7.7 minutes and the time course of changes is not stated. Künzel et al.[5] noted that there was only a drop in heart rate with umbilical venous occlusion when the fetal arterial oxygen saturation was <40%. De Haan et al.[6] noted that there was a delayed drop in heart rate with umbilical venous occlusion.

Fetal arterial blood pressure changes during umbilical cord occlusion. With umbilical arterial or umbilical cord occlusion involving all vessels, there is an increase in arterial blood pressure.[4, 6, 7, 9, 10, 12] De Haan et al.[6] noted that bradycardia occurred within 1 to 2 beats of cord occlusion, in close association with the hypertensive response.

With umbilical venous occlusion, there tends to be a decrease in fetal arterial blood pressure.[4, 12] James et al.,[11] however, noted a decrease in arterial blood pressure only with partial, mild occlusion of the umbilical vein and an increase in arterial blood pressure with complete occlusion in anesthetized baboons.

A number of workers over the years have theorized that partial occlusion of the whole umbilical cord will initially compress the vein but not the artery, because of the lower blood pressure within the vein. Then more complete occlusion is presumed to effect the arterial flow as well. While this is a reasonable theory, it has not yet been demonstrated with differential flows in the artery and the vein during partial and complete compression maneuvers.

The majority of studies have involved cord occlusion for relatively brief periods. Few studies of cord occlusion have been carried out for prolonged periods of up to and more than 1 hour. At moderate degrees of occlusion (33% reduction of control umbilical arterial flow) Itskovitz et al.[7] demonstrated tachycardia. With increased occlusion (67% reduction of flow) hypertension with a decrease in FHR was noted; FHR recovered after 15 minutes. In contrast, Ball et al.[9] occluded the cord to produce severe fetal hypoxemia with arterial oxygen saturations <10% and with adjustment of the degree of occlusion to prevent catastrophic falls in arterial blood pressure. In these studies the aim was to reduce cerebral oxygen consumption to a point where fetal brain damage would occur. Preliminary studies suggest that fetal seizure activity, as an index of fetal brain damage, occurs when the initial hypertension produced by cord occlusion reverts to control values. This is in contrast to fetal hypoxemia produced by uterine arterial occlusion.[14] Under these conditions a fetal hypotension of approximately 10 mm Hg below control values always occurred when there was subsequent seizure activity.

Arterial blood gas changes during umbilical cord occlusion. Most workers report hypoxemia, hypercarbia and acidosis with either complete or partial umbilical cord occlusion,[5, 7, 11] when measurements were made. In some cases the degree of blood gas and acid-base abnormality has been used as an index of the degree of occlusion.[8, 10]

Itskovitz et al.[15] documented the influence of cord occlusion on umbilical arterial and venous oxygen contents; even with reductions of blood flow of 80% by cord occlusion there is no reduction in umbilical venous oxygen content, whereas umbilical arterial oxygen content is reduced by >60%. Similar observations can be made in human newborns after a series of severe variable decelerations or prolonged bradycardias. Presumably in these latter cases there is reduced umbilical blood flow, allowing time for the sluggishly moving blood to equilibrate with maternal blood in the placenta.

Although data on arterial blood gases during human cord occlusion are not available, there are some data on fetal scalp blood gases taken during variable deceleration complexes during labor.[16] There is a decrease in pH by the end of a variable deceleration, but this resolves over the next several minutes. The acidosis is primarily due to a concomitant elevation of carbon dioxide.[17]

The role of the autonomic nervous system in heart rate and blood pressure changes during umbilical cord occlusion. Since the initial work of Barcroft[3] showing that vagotomy altered the initial bradycardia produced by cord compression, thus demonstrating the role of the vagus in producing the bradycardia, a number of other workers have confirmed this either by surgical[4] or pharmacologic vagotomy.[6, 13] Investigators have also concerned themselves with whether the initial bradycardia is caused by chemoreceptor influence (because of the hypoxemia) or whether the baroreflex is responsible because of the rapid increase in arterial blood pressure. Itskovitz et al.[7] make a good case for the bradycardia during partial cord occlusion being initially due to the chemoreflex and later being due to the baroreflex, because there was a decrease in fetal oxygen content but no specific blood pressure elevation for about 30 seconds.

In considering the mechanical changes during cord occlusion, in compression of the umbilical artery there will be an increase in resistance, and this will manifest

as an increase in arterial blood pressure. Should there be only partial cord occlusion, with compression of the umbilical vein but not the artery, then there will be a decrease in venous return to the heart. This may result in a fetal hypoxemia that could then be responsible for a chemoreceptor-produced bradycardia. As noted above, however, it is impossible to know in any particular situation of partial cord occlusion what the differential arterial and venous umbilical blood flows are because this has not yet been measured.

There has been some work on the role of the sympathetic nervous system in the cardiovascular changes produced by umbilical cord occlusion. De Haan et al.[6] used α-adrenergic blockade during umbilical cord occlusion and found that there was still an initial increased blood pressure but that this decreased subsequently. In umbilical cord occlusion in anesthetized baboons, James et al.[11] noted that with partial mild occlusion there was an increase in FHR, which was prevented by sympathetic blockade with propranolol (a β-adrenergic blocker) or phentolamine (an α-adrenergic blocker). From the evidence presented one could conclude that during the vagally-produced bradycardia there is simultaneous sympathetic chronotropic activity that is less and that this is masked by the intensity of the vagal bradycardia.

Amnioinfusion and other clinical evidence of cord occlusion. There have been attempts to correlate umbilical cord position at delivery with the presence of variable decelerations in labor. In one study population,[18] 28% of fetuses were found at delivery to have a cord position that was felt to be susceptible to compression. However, there was no convincing evidence that the incidence of variable decelerations was greater in the group with "abnormal" cord position. The authors described a heart rate pattern consisting of accelerations and variable decelerations, and this finding was significantly associated with an abnormal cord position. They felt that these changes occurred with mild occlusion of the cord.

Gabbe et al.[19] documented the association of oligohydramnios and variable decelerations in a chronic rhesus monkey preparation and felt this was caused by cord compression. When amniotic fluid volume was replaced through an indwelling catheter, the variable decelerations were virtually abolished. The logical extension of this work was to investigate whether replenishing amniotic fluid in the uterine cavity in humans would reduce the incidence of variable decelerations. In 1985 Miyazaki and Nevarez[20] reported on the results of a randomized trial of amnioinfusion in patients with variable decelerations in labor. With an infusion of 800 ml of normal saline solution over 40 to 60 minutes, variables were abolished in 51% of patients compared with 4.2% that resolved spontaneously in the nontreatment group. Nageotte et al.[21] in the same year also found amnioinfusion in preterm fetuses to be successful. They randomized a group of patients with preterm premature rupture of membranes to treatment with amnioinfusion or control during labor. They documented a significantly lower incidence of variable decelerations in the treated group. The premise is that amnioinfusion provides renewed cushioning for the umbilical cord, protecting it from compression. Its apparent success provides indirect evidence for the role of cord compression in the origin of variable decelerations.

The influence of fetal head compression

It has been known for over 150 years that compression of the fetal vertex appears to be associated with decelerations in heart rate. It has been speculated that compression of the brain leads to "activation of vagal centers."[22] In 1933 Rech[23] elicited fetal bradycardia that mimicked variable decelerations of varying severity by adjusting the force of the squeeze with his hand on the fetal vertex. Chung and Hon[24] refined this by placing hard pessaries in the vagina of women in advanced labor. In between contractions pressure was exerted on these pessaries, compressing the fetal vertex. This led to variable-type decelerations in 18 of 19 attempts. They felt this was similar to the pressure exerted by the dilating cervix. Further evidence in their minds for vagal stimulation by head compression was the consistent decelerations noted with the locking of forceps and initiation of traction.

Paul et al.[25] studied this phenomenon in anesthetized fetal sheep. Manual compression of the fetal vertex was performed for 20 to 50 seconds. Carotid blood flow decreased markedly with the compression, and blood pressure increased as profound bradycardia ensued. The investigators believed that this apparent vagal response could be either baroreceptor or chemoreceptor mediated. Mann et al.[26] used a metal clamp to apply pressure to fetal sheep heads. They found no consistent changes in heart rate, but cerebral blood flow and metabolism decreased as did cerebral oxygen consumption.

The fact that variable decelerations cannot be effectively abolished by amnioinfusion in the second stage of labor supports the view that head compression is a mechanism for variable decelerations.[21] Kelly[22] speculated that bradycardia with head compression was an appropriate physiologic response. With increased diastole, according to him, blood flow through the foramen ovale and the ductus arteriosus changes so as to shunt more oxygenated umbilical venous blood to the brain. In contrast, Goodlin and Haesslein[27] suggested several additional mechanisms for variable decelerations, including fetal Valsalva maneuver and maternal activities

such as voiding and seizures. They felt that some of the bradycardias may be "adult" reflexes that are inappropriate for the fetus and may result in reduced fetal blood flow and oxygenation.

Cardiac output regulation

It has commonly been stated that in the fetus the cardiac output is closely related to heart rate. In adults the beat-to-beat amount of blood pumped by the heart is determined by the amount of blood returning to the heart. In accordance with the Frank-Starling mechanism, when the cardiac muscle is stretched before contraction by an increased inflow of blood, it contracts with a greater force than before and is able to pump out more blood. In other words, the heart pumps all the blood that flows into it without excessive damming of blood in the veins. This mechanism has been studied in unanesthetized fetal lambs and has been shown to be less developed than in adult sheep. The imperfect mechanism in the fetal lamb is probably due to the fact that the heart muscle is not as well developed as in the adult sheep. It is likely that the same is true of a human fetus, as it is generally more immature than a fetal lamb when born. As a consequence, increases in the filling pressure or preload produce minor, if any, changes in combined ventricular output, suggesting that the fetal heart normally operates near the top of its function curve.

The relationship between FHR and cardiac output has not been confirmed in human fetuses under physiologic conditions, because the spontaneous increase in heart rate has been found to be associated with a decrease in stroke volume, maintaining the cardiac output unchanged.[28]

In addition to heart rate and preload, cardiac output depends on afterload and intrinsic contractility. The fetal heart appears to be very sensitive to changes in the afterload, represented by the fetal blood pressure. In this way, increases in afterload dramatically reduce the stroke volume or cardiac output. As has already been stated, the fetal heart is incompletely developed compared with that of adults. Many ultrastructural differences between the fetal and adult heart account for a lower intrinsic capacity of the fetal heart to contract. Each of these four determinants of cardiac output do not work separately, but rather they interact dynamically to modulate the fetal cardiac output during physiologic conditions.[29]

On the basis of clinical observations, it appears that the fetus can generally tolerate decelerations or bradycardias that are not <80 beats/min presumably because of the above compensatory interactions. However, at severely reduced heart rates, particularly <60 beats/min, it is likely that fetal cardiac output, and hence umbilical blood flow, cannot be maintained. Thus a deceleration of heart rate <60 beats/min either due to cord occlusion or reduced fetal cardiac output, will signify insufficiency of umbilical blood flow.

Evidence from Doppler velocimetry studies

In recent years, the ratio of systolic-to-diastolic velocity profiles in various fetal and maternal vessels has been used as an index of vascular resistance. That is, when the systolic/diastolic ratio increases, increased vascular resistance is one of the causes, and this has been used to indicate fetal hemodynamic compensations. It is now quite clear that a change in vascular resistance is only one of the components giving rise to changes in systolic/diastolic ratios, other features being a prolonged cardiac cycle, compliance of the vascular system, kinetic reflection of the velocity wave, and possibly other factors.[30]

In fetal sheep the systolic/diastolic ratio has been studied during short-term occlusion of both the umbilical vessels and the uterine artery.[8] During total umbilical vessel occlusion for 60 seconds there was a bradycardia and an increase in the systolic/diastolic ratio. The heart rate changes mimicked variable decelerations. The systolic and diastolic velocities both fell, but the diastolic velocity fell more than the systolic, leading to an increase in the systolic/diastolic ratio. The authors speculated that the fall in diastolic velocity was an indication of increased downstream resistance occasioned by the umbilical arterial compression and that the fall in systolic velocity was evidence of pump compromise. In contrast to this, uterine artery occlusion produced fetal hypoxemia, with a decrease in both systolic and diastolic velocities giving little change in the systolic/diastolic ratio. The FHR change mimicked late decelerations. The authors speculated that this change was produced not by an increase in resistance to perfusion on the fetal side of the placenta but was consistent with a decrease in fetal cardiac output and umbilical blood flow.

Weiss et al.[31] studied eight women in labor all of whose fetuses were demonstrating variable decelerations. They were in the active phase of labor with cervical dilatation between 2 and 7 cm. The authors describe a number of technical difficulties in measuring the velocity profiles in these fetuses and freely discarded invalid data. In six of the eight women there was an increase in the systolic/diastolic ratio with reversed diastolic flow, indicating that there was no net forward flow. The increase in the systolic/diastolic ratio began before the decrease in FHR. The authors concluded that this was consistent with the general theory of mechanical obstruction of the umbilical cord producing an increase in afterload and resistance, resulting in the bradycardia. In the other two of the eight women studied there was an increase in the systolic/diastolic

ratio, but this could be explained on the basis of a heart rate effect. If the systolic/diastolic ratio was corrected for that prolonged cardiac interval due to the bradycardia, there was no change in the systolic/diastolic ratio. The authors felt that this latter pair of fetuses had a moderate decrease in flow due to local or systemic hypoxemia of the fetus. The implication is therefore that these fetuses did not have variable decelerations due to cord compression but rather some other vagal activity, possibly due to hypoxemia.

Comment

Variable decelerations are vagally mediated. The vagal response may be due to either chemoreceptor or baroreceptor input. Possibly the initial deceleration (that is, the first seconds) may be due to chemoreceptor activity and the persistence of the deceleration due to baroreceptor activity.

Cord occlusion is a likely cause of many variable decelerations. We theorize, however, that this is particularly true of the first stage active phase of labor. Cord occlusion, either partial or complete, can cause both increases in afterload and decreases in fetal arterial oxygen content, both of which will result in the vagal activity causing the bradycardia. There may also be a sympathetic discharge, both α- and β-adrenergic, responsible for the hypertension in the case of the former and tendency toward increased heart rate in the case of the latter. However, the vagal influence is greater and, therefore, the net result of this is a deceleration.

Head compression is a likely cause of many variable decelerations. We theorize that this is particularly true in the second stage of labor, when cord compression is unlikely in many cases. Head compression causes a vagal discharge due to dural stimulation. This vagal discharge causes a bradycardia. If this is sufficiently severe the bradycardia may result in decreased fetal cardiac output and umbilical blood flow due to the relatively poorly developed Frank-Starling mechanism at low heart rates in the fetus.

In the case of mild to moderate variables (that is, with the FHR not <60 beats/min), the significance of these purely vagal decelerations may well be less than that of the cord occlusion variable decelerations. As yet there is no information regarding fetal acid-base changes with the two types of variable decelerations. Such data could be gained by fetal blood sampling during two types of variable decelerations as distinguished by Doppler velocimetry.

Distinguishing the types of variable decelerations may have important practical significance, because if it can be shown that the head compression decelerations are more benign, this would allow more conservative management of the second stage of labor, where they probably most frequently occur.

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Discussion

DR. E. PAUL KIRK, Portland, Oregon. This is an elegant review of the current understanding of the physiologic mechanisms of variable decelerations. Continuous electronic FHR monitoring has been with us now for more than two decades, and the fact that it has become a standard part of obstetric practice has in no way lessened our need to understand the physiologic basis for the patterns that we see and react to on a daily basis. Our ambivalence toward the use of continuous monitoring is characterized by the observation that its widespread use has been accompanied on the one hand by a rise in cesarean section rates with little apparent impact on long-term sequelae and on the other hand by the virtual elimination of intrapartum death and a vast personal and anecdotal experience where fetal monitoring appears to have been invaluable in individual cases.

Continuous electronic FHR monitoring is part of our lives on the labor and delivery floor. Fifty percent of tracings show some abnormality, and the challenge has to be not only to accurately report what we see but also to strive to understand what we are seeing and to understand the etiology of abnormalities and their physiologic basis so that the tracing can be intelligently interpreted in the context of the individual circumstance and within the perspective of the particular stage of labor. Dr. Parer has been in the vanguard of the movement, demanding intelligent interpretation of, rather than blinkered reaction to, abnormal tracings, and this paper represents another excellent contribution in that genre.

The authors describe the observed effects of partial and complete occlusion of the arterial and venous umbilical vessels, namely, an abrupt bradycardia of variable duration. They describe the consequences in terms of the blood pressure responses and the production of hypoxemia, hypercarbia, and acidosis. They recall the early work that demonstrated the effect of medical and surgical vagotomy and the suggestions of an early baroreceptor and later chemoreceptor effect. They draw some analogies from clinical observations in the second stage of labor and the effects of amnioinfusion. They emphasize the relatively incomplete development of the fetal heart and its sensitivity to afterload changes, but they seem unable to draw any convincing conclusions from Doppler velocimetry studies. Finally, they reconcile the data by reaffirming two separate etiologies for variable deceleration, making a likely association of first-stage events with cord occlusion and second stage events with head compression, and suggesting a practical importance in attempting a distinction between the two types of variable decelerations.

The obvious response is to ask how those distinctions might be made. I believe that the practice obstetrician is often frustrated by the apparent difficulty in bridging the gap between the evidence provided by animal laboratory experimentation and the realities of the labor and delivery suite. Could Dr. Parer suggest some further area of investigation so that the distinctions might be more easily made? Might we expect more helpful information as Doppler velocimetry techniques become better refined and might we learn something from observing intrapartum breathing patterns, given that the midbrain is the site of reception and integration of chemoreceptor and baroreceptor inputs and that the cardiovascular centers in the pons and medulla are heavily influenced by activity in the respiratory centers. The authors appear to place some weight on the observation that the fetal stroke volume lies at the breakpoint of the function curve and implies that this is a unique circumstance, although it is my understanding that the supine adult as opposed to the upright adult shows the same curve as the fetus. Finally, is it fair to describe the sensitivity of the fetal heart to afterload charges as applying to both ventricles? Work by Thornburg at our institution showed this sensitivity to apply to the right ventricle, whereas the left ventricle which supplies most of the blood flow to the brain maintained its stroke volume in the face of elevated aortic pressures.[1, 2]

Those of you who appreciate my inadequacies as a physiologist will be suspicious of the detail of some of these questions and will recognize the hand of my colleague, Dr. John Bissonnette, in their inquiry. Perhaps that is an appropriate reminder as to how dependent we journeymen clinicians are on the insights provided by fetal physiologists such as Dr. Parer. I look forward to the next article in the hope that the author will make the ultimate distinction between significant and insignificant variable decelerations.

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Dr. Robert Goodlin, Placerville, California. The fetal baroreflex has been studied and reviewed for more than 100 years, but little new information has been added in the past 25 years.[1] Besides producing acute fetal bradycardia, vagal stimulation also increases fetal beat-to-beat variability. I have wondered why during severe acute fetal bradycardia, fetal beat-to-beat variability is often not increased, but sometimes is even decreased. Does this have any prognostic significance as to fetal health? Because Dr. Parer mentioned my article on inappropriate fetal bradycardia, I wonder if he too is now willing to inhibit apparently detrimental fetal vagal bradycardia with atropine?[2] Because amnioinfusion is much less efficient than atropine in inhibiting severe fetal bradycardia, I also wonder if amnioinfusion really inhibits vagal fetal stimulation.

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Dr. Parer (Closing). I would first like to make a semantic comment about abnormal FHR patterns. We don't really know what an abnormal pattern is. Mild variable decelerations do not cause acidosis and also can hardly be called abnormal. Vagal reflex late decelerations that do not cause acidosis should not be termed abnormal. A better term might be "variant" heart rate patterns.

Regarding the cardiac output of the fetus during bradycardia, it is possible that the right and left ventricles may have different responses, as Dr. Kirk has pointed out. He notes that maintenance of left ventricular output under these conditions would be valuable for maintaining blood flow to the brain and heart. Although such data are not yet available for the human under such specific conditions as second stage variable decelerations, this could be an explanation why they may be a more benign process for the fetus than cord compression variable decelerations.

Dr. Kirk asks how we may clinically distinguish between variable decelerations produced by cord compression and the purely vagal type. I would like to think that Doppler velocimetry holds the key. However, when I look at the work of Weiss et al. and what they had to go through to get data on eight patients and the amount of information they had to discard to get clean studies, I would think that the journeyman obstetrician would be unable to get clinically useful observations in the second stage of labor. The technical difficulties are too great. Maybe it is sufficient to maintain that second stage patterns are these that are purely produced by the vagus nerve and that we should be more conservative in our approach to the second stage variable deceleration. That is, we should not cry fetal distress and put the forceps on after a brief period of such variable decelerations. It is a clinical observation that the well-grown fetus at term can tolerate, in the vast majority of cases, at least 30 minutes of severe variable decelerations. If purely of the vagal type, they may be tolerated for a lot longer. Additionally, in a clinical setting you would be able to rescue the decompensating fetus in a reasonably quick period of time, either by an operative vaginal delivery, by discouraging pushing with every contraction, or by short-term tocolysis. I hope that further work will strengthen the concept of the two causes of variable decelerations and that some technique for clinically distinguishing them will emerge.

Dr. Goodlin wonders that if there is an increase in vagal activity during these variable decelerations in the second stage, can we detect an increase in FHR variability compared with first-stage variable decelerations? To my knowledge that has not been shown. In any case, I am not sure that an increase in vagal activity will cause an increase in variability. Rather, the vagus is transmitting increased activity from another source. However, differences in variability in the two types would be worth looking at.

I am not yet ready to give these patients atropine in the second stage. The data are not yet available to show that such treatment is beneficial. Another problem is that once you have given the atropine, the effect remains for a long time, and you lose the heart rate tracing that you are using to follow the condition of the fetus. You can get into multipharmacology obstetrics and give drugs to subsequently counteract the atropine, but I think that is going too far.

As I noted earlier, amnioinfusion was not as effective in the second stage as it was in the first stage.

Elective induction versus spontaneous labor: A retrospective study of complications and outcome

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OBJECTIVE: This retrospective study was undertaken to identify whether electively induced labor places the mother or her fetus at increased risk as compared with her spontaneous labor cohort.
STUDY DESIGN: This study compares the labor, delivery, and fetal outcome of 253 parturients in which labor was electively induced with 253 matched controls who began labor spontaneously. All patients were delivered at Huntington Memorial Hospital in Pasadena, California, during the calendar year 1990. The patients were between 37 and 41 weeks' gestation and had no medical or obstetric conditions necessitating induction. The cases and controls were matched for age group, parity, gestational age, and delivering obstetrician.
RESULTS: Epidural anesthesia was used in 83.8% of patients in the induction group versus 55.7% in the spontaneous group. Patients with labor induced had a shorter first stage of labor. Meconium staining was found significantly more often in the spontaneous group than in the induced group (16.2% vs 6.7%). This contributed to a greater rate of neonatal consultations in the spontaneous labor group. Although cesarean section rates between the two groups were similar, nulliparous patients in the induction group with an estimated Bishop score of ≤5 had a 50% cesarean section rate. Iatrogenic prematurity was not encountered. No differences existed between the two groups with respect to intrapartum maternal complications, fetal complications, or postpartum complications.
CONCLUSIONS: When compared with spontaneous labor, elective induction of labor at term does not appear to pose an increased risk to the mother or her fetus in a carefully selected patient population. However, elective induction of labor in a nulliparous patient with an unfavorable cervix should be discouraged. (AM J OBSTET GYNECOL 1992;166:1690-7.)

Key words: Elective induction, induction, oxytocin, meconium staining, epidural anesthesia

Induction of labor is a valuable and important clinical tool in obstetrics. It is a well-accepted therapy when initiated for a medical or obstetric indication. When induction of labor is done for convenience or psychosocial reasons, it is elective and is controversial.

The rate of induction is on the rise,[1, 2] with an increasing number of obstetricians and their patients choosing to electively induce labor. At Huntington Memorial Hospital, a tertiary care center, 30% of the inductions done in 1990 were done electively without obvious medical or obstetric indications.

Although the literature on elective induction is limited, advantages and disadvantages have been described.[3-5] Opinions differ regarding the risk to mother and fetus during elective induction of labor.[1, 4-7] This retrospective study was undertaken not to validate or promote elective induction but rather to identify whether electively induced labor places the mother and her fetus at increased risk as compared with her spontaneous labor cohort.

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Patients and methods

The patients in this study were delivered at Huntington Memorial Hospital in Pasadena, California, during the calendar year 1990. Huntington Memorial Hospital is a private tertiary care facility with 5561 deliveries during the year 1990.

The patients in the study group were identified by examining hospital lists of patients undergoing elective induction of labor. The charts were then individually reviewed to determine eligibility. Patients selected were required to have a singleton, cephalic presentation with a gestional age between 37 and 41 weeks without any medical or obstetric conditions necessitating induction. The exclusion criteria are listed in Table I. This resulted in an elective induction group of 266 patients.

Each patient in the induction group was then individually matched with a patient with the same eligibility criteria and whose labor had begun spontaneously. They were matched for age (±4 years), parity, gestational age (±1 week), and delivering obstetrician. The

Table I. Exclusion criteria for study and control groups

Obstetric
Previous cesarean section or uterine scar
Multiple gestation
Noncephalic presentation
Fetal growth retardation
Spontaneous rupture of membranes, not in labor
Postdates
Oligohydramnios
Polyhydramnios
Abnormal antepartum testing
Use of tocolytic therapy within 4 wk before labor
Medical problems
Pregnancy-induced hypertension and preeclampsia
Gestational and type I diabetes
Chronic hypertension
Hemoglobin <10 gm/dl
Any serious illness or medical problem (e.g., epilepsy, cardiac diseases)

spontaneous labor control group was chosen from hospital lists of patients with spontaneous labor. The first suitable match was taken in each case by a person independent of this study. No match could be found for 13 of the patients undergoing labor induction; they were therefore excluded. This left 253 patients in each group.

For the purpose of this study, induction was defined as the initiation of labor in a woman with intact membranes and no regular uterine contractions. A Bishop score[8] of ≤5 or >5 was estimated on each patient in the induction group. This was based on the patient's initial recorded cervical examination before the induction was started. The methods of induction could include artificial rupture of the membranes, oxytocin infusion, prostaglandin E_2 gel or suppositories, or any combination thereof. Spontaneous labor in the control group was defined as the onset of regular uterine contractions 5 minutes apart with or without rupture of membranes. The number of patients in the control group requiring oxytocin augmentation was also recorded.

The data gathered on all 506 patients were obtained through extensive review of patient records. This included the labor, delivery, and postpartum records and nursing notes and parturition summaries dictated by the obstetrician. Labor abnormalities were defined by Friedman's criteria.[9] Fetal distress was defined as an abnormality of fetal heart rate necessitating that the obstetrician terminate labor by assisted vaginal delivery or cesarean section.

The data were entered into a computer and the BMDP and EPI-INFO softwares were used for statistical analysis. The paired t test was used to compare groups for continuous variables. The odds ratio analysis for case control study was used to compare groups for discrete variables.

Table II. Comparison of matching criteria between groups

	Induction group (N = 253)	*Spontaneous group (N = 253)*
Maternal age (yr)		
Average	29.90	29.85
SD	4.92	4.67
Parity (%)		
Nulliparous	30.4	30.4
Multiparous	69.6	69.6
Gestational age (wk)		
Average	39.43	39.32
SD	1.02	0.90

Table III. Method of induction

Type	*No.*	*%*
Oxytocin	213	84.2
Oxytocin plus artificial rupture of membranes	21	8.3
Artificial rupture of membranes	6	2.4
Prostaglandin	4	1.6
Prostaglandin plus oxytocin	9	3.6
TOTAL	253	100.0

Results

Study group profile. The patient profiles of both groups were comparable for age, parity, and gestational age, as shown in Table II. A total of 26 obstetricians was represented. The number of pairs per physician varied from one (0.4%) to 28 (11.1%). Twelve of the 26 physicians contributed 82.2% of the patients.

Induction profile. Oxytocin alone or in combination with artificial rupture of membranes was the method of induction used in 234 of the 253 patients (92.5%) (Table III). Within the induction group, 24.1% (61/253) of the patients had an estimated Bishop score of ≤5, and 75.9% (192/253) had a score of >5.

Labor. The length of the first stage of labor was shorter in the induction group than in the spontaneous labor group, 6.0 versus 7.2 hours ($p = 0.008$). No differences in duration of the second stage of labor were found between the groups. The duration of ruptured membranes was also similar between the two groups, 5.6 hours for the induction group and 5.0 hours for the spontaneous labor group (Table IV). In addition the numbers of reported vaginal examinations during labor were similar, 5.2 (±2.1) for the induced and 5.0 (±2.1) for the spontaneous labor patients. In the spontaneous labor group 40% of the patients received oxytocin augmentation.

The use of epidural anesthesia was statistically different between the two groups. In the induction group, 83.8% (212/253) used epidural anesthesia, whereas 55.7% (141/253) used epidural anesthesia in the spontaneous labor group ($p < 0.001$) (Table V).

Table IV. Duration of first and second stages of labor and ruptured membranes

	*No. of pairs**	*Induction group (mean ± SD)*	*Spontaneous labor group (mean ± SD)*	*Significance*
First-stage labor (hr)				
Total	196	6.0 ± 3.1	7.2 ± 5.2	$p = 0.008$
Nulliparous	42	7.2 ± 3.2	9.9 ± 5.1	$p = 0.007$
Multiparous	154	5.7 ± 3.0	6.4 ± 5.0	NS
Second-stage labor (min)				
Total	196	44 ± 61	39 ± 44	NS
Nulliparous	42	92 ± 51	90 ± 53	NS
Multiparous	154	31 ± 56	26 ± 30	NS
Duration of ruptured membranes (hr)				
Total	253	5.6 ± 3.6	5.0 ± 5.3	NS
Nulliparous	77	7.7 ± 4.3	7.6 ± 5.5	NS
Multiparous	176	4.6 ± 2.9	3.9 ± 4.9	NS

NS, Not significant.
*Cases in which cesarean section was performed were omitted in calculation of the first and second stages of labor.

Table V. Comparison of mode of delivery between two study groups

Mode of delivery	*Induction group*		*Spontaneous group*		*Significance*
	No.	*%*	*No.*	*%*	
All patients	253		253		
Primary cesarean section	37	14.6	28	11.1	NS
Vaginal forceps and vacuum	70	27.7	53	20.9	NS
Spontaneous vaginal delivery	146	57.7	172	68.0	$p = 0.02$
Cesarean section, vaginal forceps and vaccum	107	42.3	81	32.0	$p = 0.02$
Patients receiving epidural anesthetic	212	83.8	141	55.7	$p = 0.001$
Cesarean section, vaginal forceps and vacuum	101	47.6*	68	(48.2)*	NS
Spontaneous vaginal delivery	111	52.4*	73	(51.8)*	NS

NS, Not significant.
*Percent of total patients receiving epidural anesthetic.

Table VI. Percentage distribution by mode of delivery for subgroups defined by parity

	Induction group	*Spontaneous group*	*Significance*
Nulliparous			
No. of cases	77	77	
Primary cesarean section (%)	33.8	22.1	NS ($p = 0.15$)
Forceps-assisted plus vacuum-assisted vaginal delivery (%)	36.4	31.2	NS
Multiparous			
No. of cases	176	176	
Primary cesarean section (%)	6.2	6.2	NS
Forceps-assisted plus vacuum-assisted vaginal delivery (%)	23.9	16.5	NS

NS, Not significant.

Delivery. The primary cesarean section rate was 14.6% for the induction group and 11.1% for the spontaneous group (Table V). The assisted vaginal delivery (vaginal forceps or vacuum) rate for the induction group was 27.7% and 20.9% for the spontaneous labor group. There was a significant difference in spontaneous vaginal delivery rates between the two groups, 57.7% for the induction and 68% for the spontaneous labor group ($p = 0.02$). This translates to a significantly higher combined assisted vaginal delivery and cesarean section rate for the induction group than for the spontaneous labor group (42.3% vs 32.0%, $p = 0.02$). However, when only patients receiving epidural anesthesia in both groups were considered, the rate of combined cesarean section and assisted vaginal deliveries was nearly identical, 47.6% (101/212) in the induction group and 48.2% (68/141) in the spontaneous labor group (Table V).

When the role of parity and the mode of delivery were analyzed, the nulliparous patient in the induction group had an indication toward a higher cesarean section rate than the nulliparous patient in the spontaneous group, 33.8% compared with 22.1% ($p = 0.15$). The multiparous cesarean section rates were the same, 6.2% (Table VI). No statistically significant differences were found in the rate of assisted vaginal delivery with respect to parity (Table VI).

The nulliparous patient with a Bishop score of ≤5

Table VII. Cesarean section deliveries by parity and Bishop score

Parity	*Induction group (Bishop score ≤5)*	*Induction group (Bishop score >5)*	*Spontaneous group*
Nulliparous	50.0% (12/24)	26.4% (14/53)	22.1% (17/77)
Multiparous	5.4% (2/37)	6.5% (9/139)	6.2% (11/176)

Numbers in parentheses indicate numerator and denominator of percent.

had an indication toward a higher cesarean section rate than those with a Bishop score >5, 50% compared with 26.4% ($p = 0.12\%$). The cesarean section rate for the nulliparous patient in the spontaneous labor group was 22.1%. The Bishop score did not appear to affect the cesarean section rate for the multiparous patient (Table VII).

The indications for a cesarean section were similar for both groups with the exception of the indication "primary arrest of dilatation." The induction group had five cesarean sections done for this indication, whereas the spontaneous group had none ($p < 0.01$ on the basis of the Poisson probability) (Table VIII). All five of the induction patients with primary arrest of dilatation were nulliparous with a Bishop score of ≤5.

The time of day that the deliveries occurred in the spontaneous labor group was evenly distributed over the 24-hour period. The induction group, however, had only 11% of its deliveries between midnight and eight AM (Fig. 1).

Conditions of fetus and newborn. All babies were born alive, and there were no neonatal deaths. There was no iatrogenic prematurity in the induction group. Fetal outcome is shown in Table IX. The mean birth weight of infants in the induction group was 3553 gm compared with 3434 gm in the spontaneous group ($p = 0.0007$). There were 33 babies whose birth weight was >4000 gm in the induction group and 21 in the spontaneous group.

There was no significant difference between the two groups in the number of cases with Apgar scores <7 at 1 or 5 minutes. Additionally, there was no difference in fetal heart rate abnormalities or other fetal complications. Meconium was found in 17 patients (6.7%) in the induction group but occurred significantly more often in the spontaneous labor group, 41 patients (16.2%) ($p = 0.0013$). In addition, a neonatal consultation was obtained in 17.4% of the cases in the spontaneous labor group, as opposed to 9.1% in the induction group ($p = 0.009$). The reasons for neonatal consultations are shown in Table X. Meconium was the reason for consultation in 15 (5.9%) of the induced patients and 34 (13.4%) of the spontaneous labor patients ($p = 0.007$). There were six neonatal intensive care unit admissions in the spontaneous labor group and two in the induction group. One baby in the induction group was admitted after shoulder dystocia, severe variable decelerations, and birth asphyxia (weight 3700 gm, Apgar scores 1 and 6). The other admission was for transient tachypnea and to rule out pneumonia. The infant did not have respiratory distress syndrome. In the spontaneous labor group three admissions were for amnionitis, one for meconium aspiration, one for birth asphyxia (Apgar scores 2 and 6), and one for respiratory difficulty. All infants were discharged home from the neonatal intensive care unit in good condition.

Table VIII. Indications for cesarean section

Indications	*Induction group*		*Spontaneous group*		*Significance*
	No.	*%*	*No.*	*%*	
Primary arrest of dilatation	5	2.0	0	0.0	$p < 0.01$
Secondary arrest of dilatation	12	4.7	10	4.0	NS
Arrest of descent	11	4.3	12	4.7	NS
Fetal distress	11	4.3	8	3.2	NS
Cord prolapse	2	0.8	1	0.4	NS
Malposition	0	0.0	1	0.4	NS
Other	0	0.0	1	0.4	NS

NS, Not significant.

Note: Some physicians reported more than one indication per case.

No significant differences between the two groups were observed for lacerations, intrapartum maternal complications, or postpartum complications (Table XI).

Comment

According to the American College of Obstetricians and Gynecologists, "Induction of labor is undertaken when, in the opinion of the physician, the risks of delivery to the mother or the fetus or both are less than the risk of continuing the pregnancy."[10] Elective induction of labor is performed before there exists a known risk to the mother or fetus of continuing the pregnancy. This procedure should be considered justifiable only after it is shown that there is no increased risk to the mother or fetus and that the benefits of success outweigh the disadvantages of failure.

Opinions differ regarding the risk to mother and fetus during elective induction of labor.[1, 3-7, 11, 12] Proponents of elective induction argue that they are avoiding potential adverse outcomes associated with postdates, preeclampsia, and term intrauterine fetal death of unknown causes.[6, 12, 13] Others advocate elec-

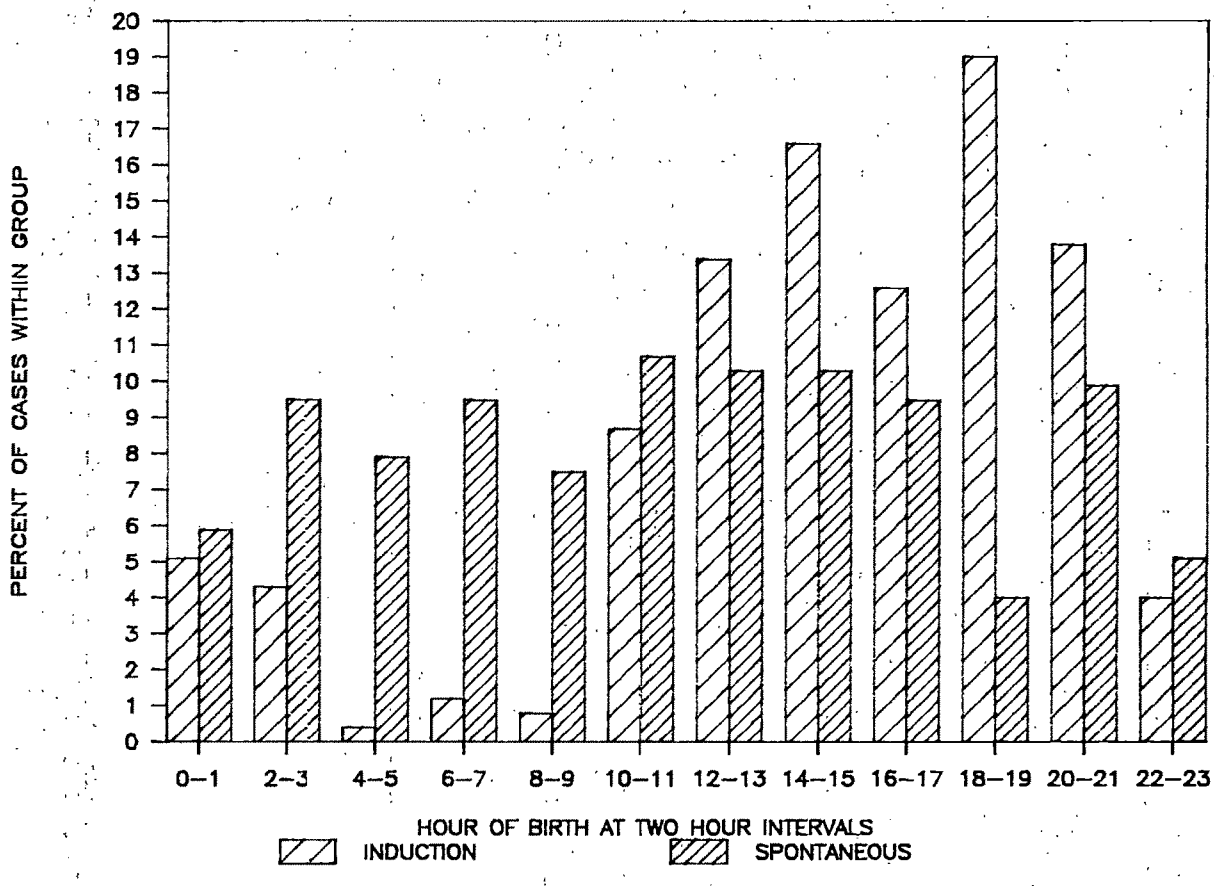


Fig. 1. Percentage distribution by hour of birth.

tive induction to allow for daytime deliveries with a rested patient and better perinatal medical care personnel.[3, 6, 14] It allows for better planning by the physician, patients, and their families. Patient anxiety may be reduced, resulting in a more pleasant birth experience by having her own physician present and planning for pain control with epidural anesthesia.

Those opposing elective induction would argue that not only is it generally not recommended by the American College of Obstetricians and Gynecologists or the Food and Drug Administration but also it is an unnecessary and unnatural process.[13, 15] There is concern over inducing labor before the fetus is mature or "ready." Smith et al.[3] expressed concern that once a physician has initiated one form of active intervention (the decision to electively induce labor) there may be a tendency to more readily accept further intervention in the form of operative or assisted deliveries.

Previous studies have cited difficulties in obtaining control groups comparable to the induction groups and controlling for differences in practice styles of individual physicians.[7, 12] In this study we felt confident that we attained a control group very similar to the induction group. All patients were considered low risk, with no medical or obstetric complications. They were matched closely for age, parity, gestational age, and physician, the latter in an attempt to minimize the physician style differences or tendencies of one physician to affect outcome of either group.

In this study the majority of the induced labor patients were delivered in the afternoon and early evening. The spontaneous labor patients were delivered evenly over the 24-hour period. Similar findings were also reported by other studies.[3, 5, 14]

One trend that appears fairly consistent throughout the literature is the increased use of epidural anesthesia in the induction group.[1, 3-5, 11] This study is consistent with previous findings, because 83.8% of induced labor patients received epidural anesthetic whereas only 55.7% of the spontaneous labor patients did so. A possible explanation may be that when patients agreed to planned or scheduled delivery many of them also planned for pain control.

The induced labor group of patients had a shorter first stage. This occurred more often in nulliparous patients.

We did not find a significant increase in the number of cesarean sections or assisted vaginal deliveries in the

Table IX. Fetal outcome

Outcome	*Induction group (N = 253)*	*Spontaneous group (N = 253)*	*Significance*
Birth weight (gm)			
Mean ± SD	3,553 ± 385	3,434 ± 437	$p = 0.0007$
Range	2722-4508	2098-4848	
Apgar score < (no.)			
At 1 min	12 (4.7%)	8 (3.2%)	NS
At 5 min	2 (0.8%)	1 (0.4%)	NS
Fetal complications (no.)			
Heart rate abnormalities*	25 (9.9%)	29 (11.5%)	NS
Fetal distress	8 (3.2%)	3 (1.2%)	NS
Abruptio placentae	4 (1.6%)	2 (0.1%)	NS
Cord prolapse	2 (0.1%)	1 (0.04%)	NS
Meconium observed (no.)	17 (6.7%)	41 (16.2%)	$p = 0.0013$
Neonatal consultation obtained (no.)	23 (9.1%)	44 (17.4%)	$p = 0.009$
Neonatal intensive care unit admission (no.)	2 (0.8%)	6 (2.8%)	NS

NS, Not significant.
*Includes bradycardia, severe variable decelerations, and late decelerations.

Table X. Indications for neonatal consultation

Indications	*Induction group (N = 253)*		*Spontaneous group (N = 253)*		*Significance*
	No.	*%*	*No.*	*%*	
Meconium	15	5.9	34	13.4	$p = 0.007$
Fetal heart rate abnormality	9	3.6	6	2.4	NS
Amnionitis	2	0.8	6	2.4	NS
Depressed infant	3	1.2	3	1.2	NS
Respiratory difficulty	0	0.0	1	0.4	NS
Neurologic	1	0.4	0	0.0	NS

NS, Not significant.
Note: Some physicians reported more than one indication per case.

Table XI. Maternal, intrapartum, and postpartum complications

Complications	*Induction group (N = 253)*	*Spontaneous group (N = 253)*
Maternal complications		
Intrapartum fever (temperature ≥100.4° F)	4	6
Uterine atony	1	2
Hemorrhage	2	0
Hypotension	1	0
Other	1	0
Lacerations		
Third- and fourth-degree perineal	19	19
Vaginal	6	14
Cervical	1	0
Postpartum complications		
Postpartum fever (temperature ≥100.4° F)	17	24
Endomyometritis	6	7
Hemorrhage	0	1
Neurologic	0	1
Other	3	0

induction group. This is in contrast to results of Yudkin et al.[4] and Vierhout et al.[5] but similar to the findings of Sande et al.[1] and Martin et al.[6]

Patients in the spontaneous labor group had a greater chance of having a spontaneous vaginal delivery as opposed to an assisted vaginal delivery or cesarean section ($p = 0.02$). However, this appears to be related to the use of epidural anesthesia rather than the induction process itself. As described earlier, when only patients receiving epidural anesthetic in both groups were considered, the spontaneous vaginal delivery rates were nearly identical, 51.8% in the spontaneous group and 52.4% in the induction group.

Our results identified a group of patients undergoing induction who are at risk of having a cesarean section. Twenty-four nulliparous patients with a Bishop score of ≤5 underwent induction. Twelve of these patients (50.0%) required a cesarean section. If the nulliparous patients' Bishop scores were >5, the cesarean section rate was similar to that of nulliparous patients in the spontaneous labor group (26.4% vs 22.1%). Although the nulliparous patient with a Bishop score of ≤5 is at an increased risk of cesarean section, the multiparous patients' cesarean section rate did not differ with Bishop scores. Smith et al.[2] also found an increased cesarean incidence in the nulliparous patient.

Five patients in the induction group underwent a cesarean section for primary arrest of dilatation. No patients in the spontaneous labor group required a cesarean section for this diagnosis. All five of these patients were nulliparous with a Bishop score of ≤5.

Meconium staining was found more often in the spontaneous labor group. This accounted for an increase in delivery room consultations by a neonatolo-

gist. However, the presence of meconium did not appear to have an adverse effect on ultimate fetal outcome. This finding of increased meconium staining is fairly consistent throughout the literature.[3, 5-6, 11-15] One might hypothesize that a fetus under stress will initiate labor and thus explain the higher incidence of meconium staining in patients in spontaneous labor. With the induced labor patient labor may have been initiated before the fetus was stressed.

Although the average birth weight in the induced group was higher, this did not appear to affect delivery outcome. Fetal size may have contributed to the physician's decision to encourage induction before 41 weeks.

The two groups did not differ in fetal outcome as reflected by similar Apgar scores and complications. However, although the difference is not statistically significant, there were more neonatal intensive care admissions in the spontaneous labor group. Similar to findings in previous studies, there was no prematurity in the induction group.[3, 5] The maternal outcome was not adversely affected by induction, as indicated by the absence of increased intrapartum or postpartum complications.

In summary, when compared with spontaneous labor, elective induction of labor at term does not appear to pose an increased risk to the mother or her fetus in a carefully selected patient population. However, elective induction of labor in a nulliparous patient with an unfavorable cervix, should be discouraged.

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Discussion

DR. JAMES D. FEARL, Portland, Oregon. What is an elective induction of labor? In this article the authors have defined it as being done for convenience or psychosocial reasons after medical or obstetric inductions are eliminated. He has retrospectively evaluated electively induced labor in the year 1990 at Huntington Memorial Hospital, where 5561 deliveries took place during the year and 30% of the inductions were elective. A list of medical problems and obstetric conditions stated in their Table I seems to include most or all the diagnoses representing the 70% of induced labors that would be considered nonelective. It was noted that macrosomia was not included in these exclusion criteria. These exclusion critera were also used to allow selection of a comparable control group of patients and to attempt to eliminate additional factors in the conduct of labor that might influence operative delivery.

The resulting 266 patients were reduced to 253 patients when no match could be found for 13 of the induction patients. One of the more common criticisms of elective induction studies in the literature is lack of a proper control group of patients. Each patient was matched with a control patient of comparable age (±4 years), parity (nulliparous or parous), gestational age (±1 week), and the same delivering obstetrician. Although possibly not important, frequency of smoking and drug use was not determined.

The differing opinions as to the acceptability of elective induction of labor on the basis of risk to mother and fetus have been debated for decades. This group of 253 patients and their well-matched controls were studied to determine whether a mother and fetus with elective induction are placed at increased risk in 1990.

Many aspects of labor and delivery were comparable in the two groups. Frequency of neonatal consultations, incidence of meconium staining, and admissions to the neonatal intensive care unit were favorable toward the induced patients.

To examine Dr. Macer's conclusion that elective induction of labor does not pose an increased risk to mother or fetus, the parameters of fetal distress and frequency of cesarean section must be looked at. The categories of fetal outcome listed as fetal complications or fetal indications for delivery by cesarean section include fetal heart rate abnormalities, fetal distress,

abruptio placentae, and cord prolapse. Although there was no statistical significance when any individual complication is compared with the controls, all categories except heart rate abnormalities were present more commonly in the induced group of patients.

Because there were 33 newborns weighing >4000 gm compared with 21 newborns in the spontaneous labor group, I would question Dr. Macer's conclusion that this did not affect the delivery outcome. If estimated fetal size contributed to the decision to induce the labor, I would not consider this an elective induction. Can you tell us how many of the ≥4000 gm infants were delivered by cesarean section? As he pointed out, the 24 nulliparous patients with an unfavorable Bishop score had a cesarean section rate twice as high as 50.0% as that of those with a favorable score, 26.4%.

Would you consider a nulliparous patient at term with an unfavorable cervix as determined by Bishop score a candidate for elective induction? I would also be interested in your comments regarding the seeming reluctance toward the use of prostaglandin gel at least in the nulliparous patient with an unfavorable cervix. In the discussion of an article I presented at the Pacific Coast Obstetrical and Gynecological Society in 1986[1] one discussant reviewed the 11 articles presented before the Society since 1931 and noted the repeated emphasis of the cervical requisites for elective induction.

I will let the words of Dr. Robert Goodlin act as the devil's advocate by quoting his comments in his formal discussion of my article regarding the Food and Drug Administration statement warning against the use of oxytocin for elective induction. "I remember with fondness the days of elective inductions when the patient's labor was induced on Friday so that the husband could take care of the children on the weekend, the obstetrician would be relatively free of weekend deliveries, and everyone was happy. It now appears that these wonderful times are available to obstetricians and their patients in Portland (Pasadena). I am curious as to how the Food and Drug Administration regulations are avoided."

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DR. EZRA DAVIDSON, Los Angeles, California. Why do you call the decision to have or not to have an elective induction an ethical question for the patient and her physician rather than a practical one?

DR. JAMES CAILLOUETTE, Pasadena, California. In medicine today, the term "managed care" is in vogue. Managed care is brought to our doorstep by the lawyers and MBAs at the demand of employers, the buyers of medical care plans. Not initiated by physicians, it is nevertheless the wave of our future. I believe that the trend in obstetrics will continue toward managed labor because traditional care for the normal woman in labor is no longer cost-effective. It is no longer feasible for individual physicians who have invested 12 years in training at a cost of hundreds of thousands of dollars to dedicate extended periods to observing one normal woman in labor. Managed labor for the normal obstetric patient is in the cards. Let us hope that it is the obstetricians who deal the hand.

DR. CLYDE VON DER AHE, Los Angeles, California. Elective inductions were being done 50 years ago. In 1943, when I was an intern at Saint Vincent's Hospital in Los Angeles, Dr. B.J. Hanley, who was to be President of this Society, would bring in for induction of labor five to seven women on most Wednesday and Saturday mornings. He would strip and then rupture the membranes and then he would use high doses of barbiturates and scopolamine followed by drip ether anesthesia. The cesarean section rate was very low. Around 6 or 7 PM it was interesting to watch the husbands who had arrived together in the morning saying to each other, "Well, what did you get?" "I have a girl." "I have a boy."

DR. MACER (Closing). Dr. Fearl, a patient admitted for induction of labor with the diagnosis of fetal macrosomia should have been excluded from this study. However, some may have had induction for this indication and the physician did not mention the reason in the chart. As you noted, there were 33 patients in the induction group whose babies weighed >4000 gm versus 21 in the spontaneous labor group. I do not have information about how many of these large infants were delivered by cesarean section.

In this retrospective study, few obstetricians chose to use prostaglandin gel or suppositories. Although the numbers are small, it appears that its use might be advantageous. Six of the 13 patients with labor induced by prostaglandins had Bishop scores of ≤5, and there was only one cesarean section in this group.

Tubal sterilization and risk of subsequent hospital admission for menstrual disorders

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OBJECTIVE: Our objective was to investigate tubal sterilization and subsequent hospitalization for menstrual disorders.

STUDY DESIGN: Automated discharge data were used in a population-based cohort study of 7253 women aged 20 to 49 years with tubal sterilization (1968 through 1983) at Group Health Cooperative of Puget Sound. Comparisons were with an age-matched cohort of 25,448 nonsterilized women and a nonmatched cohort of 5283 spouses of men with vasectomies.

RESULTS: In the sterilization cohort, 282 had hospitalization for menstrual disorders (curettage, $n = 191$; hysterectomy, $n = 66$; nonsurgical, $n = 25$). Risk of hospitalization for menstrual disorders was 2.4 times greater after tubal sterilization (95% confidence interval 2.0 to 2.9). This risk was 6.1 times greater for sterilized women aged 20 to 24 years (95% confidence interval 0.72 to 3.2). Compared with the risk for nonsterilized women whose spouses had a vasectomy, the risk was 1.6 times greater (95% confidence interval 1.3 to 2.1). Hospitalization for menstrual disorders was not more common after unipolar sterilization than after other methods, as might have been expected if the menstrual disorder was related to impaired uteroovarian circulation.

CONCLUSIONS: Tubal sterilization is associated with a greater risk of hospitalization for menstrual disorders. A biologic association is not supported by these results. (Am J Obstet Gynecol 1992;166: 1698-1706.)

Key words: Tubal sterilization, curettage, menorrhagia, metrorrhagia

Whether menstrual disorders are more common after tubal sterilization is controversial.[1] This issue is important because medical and surgical hospital admissions to manage menstrual disturbances[2] and tubal sterilization are both common.[3] We have previously reported an increased risk of hysterectomy after tubal sterilization.[4] Uterine bleeding was the indication for only 37% of the hysterectomies among the sterilized women in that report, and hospitalizations for curettage or other treatments for menstrual disorders were not considered. To clarify the relationship between tubal sterilization and first hospitalization for a menstrual disorder, we performed a large cohort study at Group Health Cooperative of Puget Sound, a nonprofit, consumer-governed, health maintenance organization located in western Washington. Characteristics of the Cooperative's population are similar to those of the rest of the community,[5] and this site offered the additional advantage of a population-based, automated data base for the over one million persons who have ever been enrolled in the Cooperative. These data enabled us to avoid the problems of other studies that were not population-based, had limited follow-up, or lacked an independent, nonsterilized comparison group.

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Material and methods

Sterilized and nonsterilized women were eligible for study at Group Health Cooperative if they were 20 to 49 years of age between 1968 and 1983. Sterilized women were identified from surgical log books (1968-1971) and from automated hospital discharge data from the Commission on Hospital Professional Activities Study (1972-1984). Methods of sterilization were classified as postpartum (all by partial tubal resection), bipolar fulguration, unipolar fulguration, and other interval techniques (primarily isthmic tubal resection or fimbriectomy). During the period of this study, 16% of the sterilized women at Group Health Cooperative had postpartum sterilizations, 36% had bipolar techniques, 20% had unipolar techniques, and 27% had other interval techniques. Women were excluded if they

has been hospitalized for a primary or secondary diagnosis of menstrual disorder at Group Health Cooperative before the reference date. The reference date was the date of sterilization for the sterilized woman, and that date was also the reference date for the nonsterilized women matched to her. Nonsterilized women were excluded if they had a hysterectomy at Group Health Cooperative before their reference date or if they were not enrolled at the Cooperative as of their reference date. A hospitalization for menstrual disorder was included if it was the first hospitalization that had a medical record code for menorrhagia, metrorrhagia, hypermenorrhea, or dysmenorrhea. Ninety-three percent of hospitalizations for menstrual disorders were related to excessive or irregular bleeding, and 7% were related to dysmenorrhea alone.

Our analysis on the risk of hospitalization for menstrual disorder after tubal sterilization was based on two principal comparisons. The first comparison from the general population of Group Health Cooperative enrollees was between all eligible women who had a tubal sterilization ($n = 7253$) and a matched comparison group of nonsterilized women ($n = 25,448$). These comparison groups are to be referred to as the sterilized and general population cohorts, respectively; and the comparison involving these two cohorts is referred to as the population comparison. The second comparison was not matched, and it concerned only women who had a spouse enrolled in Group Health Cooperative. In this second comparison, married sterilized women ($n = 4057$) were compared with nonsterilized women ($n = 5283$) whose husbands had a vasectomy at Group Health Cooperative between 1968 and 1983. These comparison groups are referred to as the sterilized spouses and vasectomy spouses cohorts, respectively; and the comparison involving these latter two cohorts is referred to as the spouses' comparison. (The 4057 sterilized spouses are a subset of the 7253 women who were sterilized at Group Health Cooperative during the period of this study.) The spouses' comparison was included because in this comparison the couples had all chosen to have no further children. Whether childbearing is ended may be related to decisions regarding hospitalization for menstrual disorders, particularly those involving a hysterectomy.

Follow-up time began 1 year after the sterilization (or a corresponding reference date for the nonsterilized cohorts) and continued until first hospitalization for menstrual disorder, disenrollment from Group Health Cooperative, hysterectomy, or Dec. 31, 1984, the end of the study period. Hospitalizations during the first year after sterilization (or reference date) were excluded because routine preoperative and postoperative evaluation could have resulted in increased opportunity for diagnosis and treatment of menstrual disorders among sterilized women soon after the procedure. This detection bias would have increased estimates of the risk for menstrual disorders soon after sterilization.

Population comparison. Because data on factors such as gravidity and prior contraception were not contained in our automated data base, it was not suitable for assessment of confounding. Although these data would have been available from a manual review of all the hospital medical records, this was not feasible given the large number of women in the population comparison (>30,000 women). We considered medical record review of a lesser number of women from a random sample of each cohort or within the context of a case-control study. To achieve adequate statistical power, both of these approaches would also have required review of an inordinate number of medical records.[6] This would have been true for a cohort study of a random sample of subjects because the outcome (hospitalization for menstrual disorders) was rare (2%). Similarly, for a case-control study the exposure (tubal sterilization) was uncommon (10%) among women at Group Health Cooperative.

Therefore a more efficient two-stage, matched cohort design was used.[7, 8] The first stage of this cohort design is characterized by matching sterilized (exposed) and nonsterilized (nonexposed) subjects on factors available in an automated data base. Our matching was based on age (within 2 years) at sterilization (or reference date), and duration (within 1 year) of enrollment at Group Health Cooperative before sterilization (or reference date) (Table I). A 4:1 matching ratio was achieved for 86% of the sterilized cohort. For 10% of the sterilized cohort the matching ratio was 3:1, and for 4% the matching ratio was 2:1.

The second stage had a nested design that involved data abstraction from the medical records of a subgroup of these matched sets. Sets without an outcome (i.e., subsequent hospitalization for a menstrual disorder) were not included because these sets would not alter estimates of the relative risk.[8] One nonsterilized woman was selected at random from each set; thus matched sets became matched pairs, each containing one sterilized and one nonsterilized woman. All such pairs with at least one woman who was hospitalized for menstrual disorders were then identified with automated data. Because there were more pairs with hospitalization for menstrual disorders than needed to achieve statistical power to detect a relative risk of 2.0, 64 pairs (64 women from the sterilized cohort and 64 women from the general population cohort) were randomly selected for chart abstraction and data collection in the second stage of this comparison.

Medical record abstraction was done by two people. One reviewed the chart and verified the sterilization

Table I. Age and duration of Group Health Cooperative membership at sterilization or reference date, according to sterilization status

Age at sterilization/reference date (yr)	*Category of women*			
	All sterilized (n = 7253) (%)	*No sterilization (n = 25,448) (%)*	*Sterilized with spouses (n = 4057) (%)*	*Nonsterilized with sterilized spouses (n = 5283) (%)*
20-29	29	30	29	34
30-34	36	34	38	37
35-39	24	23	23	19
40-44	9	11	9	8
45-49	1	2	2	3
TOTALS	100	100	100	100
Mean of duration (yr) Group Health Cooperative membership at sterilization or reference date	5.97	5.96	5.95	5.47

status. A second person (blinded as to sterilization status) noted the presence (as of the sterilization or reference date) of several potentially confounding variables including married/living with (yes, no), gravidity, parity, history of menstrual disorder (yes, no), and contraceptive method (oral contraceptives, intrauterine device, other) in use at 6 months before the sterilization or reference date.

Spouses' comparison. Our second principal comparison (spouses' comparison) had a first stage in which we used a nonmatched cohort design to compare married, sterilized women to the wives of men who had vasectomies between 1968 and 1983. Men with vasectomies were identified from pathology logs that indicated a specimen from the vas deferens. Spouses were identified from automated enrollment data.

In the second stage of the spouses' comparison, we performed a nested, case-control study with data based on medical records to obtain relative risk estimates adjusted for the same potentially confounding variables as in the population comparison. Because the number of sterilized spouses and vasectomy spouses were similar, the case-control design had maximum efficiency for this task. Simple random samples of 105 cases of hospitalization for menstrual disorders and 205 controls not hospitalized for menstrual disorders were selected without regard to sterilization status.

Statistical analysis. For analyses involving the population comparison, the Cox proportional hazards model[9] was used to obtain estimates of the relative risk of hospitalization for menstrual disorders associated with sterilization. This analysis was stratified on the matched sets used in the design (thus controlling for age and duration of enrollment at Group Health Cooperative before sterilization or reference date). For the spouses' comparison, an unmatched Cox proportional hazards model was used to obtain estimates of the relative risk, controlling only for age and duration of enrollment. In the nested studies adjustment for the additional potential confounding variables listed above (e.g., gravidity) was by conditional logistic regression in the population comparison and by unconditional logistic regression in the spouses' comparison. These potentially confounding variables were chosen because they were considered to have an influence on a woman's inherent risk of hospitalization for menstrual disorders. All p values were based on maximal probability likelihood ratio tests. Extensions of the primary Cox logistic regression models were used to test for effect modification with age and duration of follow-up.

Results

Sterilized women had higher rates of hospitalization for menstrual disorders than did general population women: 955 versus 367 (per 100,000 person years), respectively ($p < 0.001$). The corresponding relative risk (unadjusted except for matching variables) of hospitalization for menstrual disorders associated with sterilization was 2.4 (95% confidence interval 2.0-2.9, $p < 0.001$) (Table II). This risk did not vary with duration of follow-up. The effect of sterilization depended greatly on age ($p < 0.005$). Among women who were sterilized at age 20 to 29 years, the risk of hospitalization for menstrual disorders was more than five times greater among sterilized women compared with nonsterilized women. The risk of hospitalization for menstrual disorders was 1.3 to 2.9 times greater for women sterilized in other age decades (Table III). When medical record data from the second phase of the population comparison were used to also control

Table II. Incidence and relative risk of hospital admission for menstrual disorders in sterilized women compared to nonsterilized women from the general population and spouses of men with vasectomies

	No. of women with admission for menstrual disorder	*Person years*	*Incidence**	*Relative risk†*
General population comparison				
Nonsterilized cohort	340	92,469	367	Referent
Sterilized cohort	282	29,521	955	2.4 (2.0-2.9)‡
Vasectomy spouse comparison				
Vasectomy spouse cohort§	164	20,107	816	Referent
Sterilized spouse cohort‖	153	14,291	1071	1.6 (1.3-2.1)‡

*Hospital admission for menstrual disorders per 100,000 woman years.

†Adjusted for age (continuous) and duration of Group Health Cooperative enrollment at reference date. Numbers in parentheses are 95% confidence intervals.

‡$p < 0.001$.

§Nonsterilized wife of man with vasectomy.

‖Married woman with tubal sterilization.

Table III. Risk of hospital admission for menstrual disorder after tubal sterilization according to age relative to nonsterilized women in population comparison

	Sterilized women (n = 7253)		*Nonsterilized women (n = 25,448)*		
Age (yr)	*Admissions (No.)*	*Person years*	*Admissions (No.)*	*Person years*	*Relative risk**
20-24	15	1,554	9	5,118	6.1 (1.9-19.6)
25-30	71	6,840	32	20,409	5.0 (2.9-53.2)
31-34	107	10,538	106	30,701	2.9 (2.1-30.0)
35-39	57	6,952	107	21,992	1.7 (1.2-18.0)
40-44	25	2,921	73	11,840	1.3 (0.8-14.0)
45-49	7	616	13	2,409	1.7 (0.7-21.2)
p†					<0.005

*Reference date and duration of Group Health Cooperative membership at reference date were controlled for. Numbers in parentheses are 95% confidence intervals.

†*p* Value refers to likelihood that all relative risks are the same across age groups.

for potentially confounding factors (marital status, history of prior menstrual disorders, gravidity, and prior contraception) the adjusted risk of hospitalization for menstrual disorders declined to 1.5 (95% confidence interval 0.72 to 3.2 $p > 0.20$) and was no longer statistically significant (Table IV). Gravidity was strongly related to hospitalization for a menstrual disorder ($p < 0.01$).

Sterilization procedures were analyzed individually. With unipolar procedures the risk of hospitalization for menstrual disorders increased 2.1 times (95% confidence interval 1.4 to 3.2), with bipolar procedures the risk increased 2.3 times (95% confidence interval 1.5 to 3.4), with other interval procedures the risk increased 3.8 times (95% confidence interval 2.8 to 5.1), and with postpartum methods the risk increased 2.6 times (95% confidence interval 1.6 to 4.1). However, differences between sterilization procedures in risk of hospitalization for menstrual disorders were not statistically significant ($p > 0.25$).

At the first hospitalization for menstrual disorders, the ratio of women submitted directly (i.e., without preceding curettage) to hysterectomy was 1.6 times greater in the tubal sterilization cohort compared with the nonsterilized, general population cohort (Fig. 1). Among women who were hospitalized for menstrual disorders, in the sterilized cohort 68% (191/282) had curettage as initial treatment versus 75% (255/340) from the general population cohort. When all hospitalizations for menstrual disorders were considered (after the patient's first hospitalization), hysterectomy was eventually included in the treatment of 43% (121/282) of women from the sterilized cohort who were hospitalized for menstrual disorders compared with 38% (129/340) of nonsterilized women for the general population cohort.

In the spouses' comparison, sterilized spouses had higher rates of hospitalization for menstrual disorders than did vasectomy spouses: 1071 versus 816 (per 100,000 person years), respectively ($p < 0.001$). The corresponding relative risk associated with sterilization was 1.6 (95% confidence interval 1.3 to 2.1, $p < 0.001$)

Table IV. Multivariate population comparison model*† (based on matched† pairs of sterilized and nonsterilized women) to evaluate risk of hospitalization for menstrual disorders after adjustment for potential confounding variables

Variable	*Slope (B)*	*Standard error of slope*	*Relative risk*	*95% confidence interval*	*Significance‡*
Married or living with someone					$p > 0.05$
Yes	0.0	—	1.0	—	
No	0.65	0.80	1.9	0.40-9.2	
History of menstrual disorder					$p < 0.01$
No	0.0	—	1.0	—	
Yes	1.4	0.70	4.0	1.0-15.8	
Gravidity					$p < 0.01$
0	0.0	—	1.0	—	
1	1.1	1.4	3.0	0.2-45.4	
2	2.5	1.5	12.6	0.70-232	
3 or 4	2.7	1.4	14.9	1.0-229	
>5	4.4	1.6	78.7	3.2-1950	
Prior contraceptive method					$p < 0.05$
None	0.0	—	1.0	—	
Oral contraceptives	0.33	0.71	1.4	0.35-5.6	
Intrauterine contraceptive device	−1.1	1.3	0.34	0.02-4.6	
Other	1.4	0.79	4.1	0.86-19.3	
Tubal sterilization					$p > 0.20$
No	0.0	—	1.0	—	
Yes	0.41	0.38	1.5	0.72-3.2	

*This was a matched (conditional) logistic regression model based on analysis of 64 matched pairs, each containing one sterilized woman and one nonsterilized woman. One woman in each pair was hospitalized for menstrual disorders and one woman was not. (See text for explanation of sampling procedures.)

†Matching variables were age, reference date (1977 to 1981/1982 to 1983), and duration (1-year intervals) of Group Health Cooperative membership at sterilization or reference date.

‡*p* Value for exposure tests a difference in risk across categories (based on deviance test).

(Table II). The increase in hospitalizations for menstrual disorders did not vary substantially with age category or duration of follow-up. After adjustment for potentially confounding factors (history of prior menstrual disorder, gravidity, and prior contraception) in the nested case-control study, women with their first hospitalization for menstrual disorder were 2.0 times (95% confidence interval 1.2 to 3.4, $p < 0.01$) more likely to have been sterilized (Table V).

At first hospitalization for menstrual disorders in the spouses' comparison, the ratio of women submitted directly to hysterectomy was 1.5 times greater in sterilized spouses' cohort compared with vasectomy spouses (Fig. 2). Among women who were hospitalized for menstrual disorders, 67% (102/153) of sterilized spouses had curettage as initial treatment versus 79% (129/164) of vasectomy spouses. Hysterectomy was included in the eventual treatment of 59% (91/153) of sterilized spouses with hospitalization for menstrual disorder compared with 45% (73/164) of vasectomy spouses.

In the multivariate models for both the general population comparison and the sterilized spouse comparison, adjustment for potential confounders indicated an inverse relationship between age and risk of hospitalization for menstrual disorders. Regardless of the cohort, sterilized women between 20 and 29 years of age had the greatest risk for hospitalization for menstrual disorder.

Comment

We found that sterilized women are at increased risk of subsequent hospitalization for menstrual disorders. The increased risk has at least three possible explanations. (1) Tubal destruction interferes with the uteroovarian blood flow and results in altered ovarian hormone production leading to menstrual irregularity.[10] (2) Women who elect tubal sterilization for contraception are inherently greater users of other elective gynecologic surgeries (e.g., curettage, hysterectomy). (3) After reproduction is no longer possible, physicians and their patients are more inclined to choose hysterectomy as management for menstrual disorders.[11]

Under the hypothesis that impaired uteroovarian circulation results in altered estrogen production and menstrual disturbance,[10] unipolar fulguration, which disrupts more of the mesosalpinx than other sterilization techniques,[12] would be expected to have a greater risk of menstrual disturbance than other sterilization methods. However, our results indicated that the risk of menstrual disturbance was lowest among women

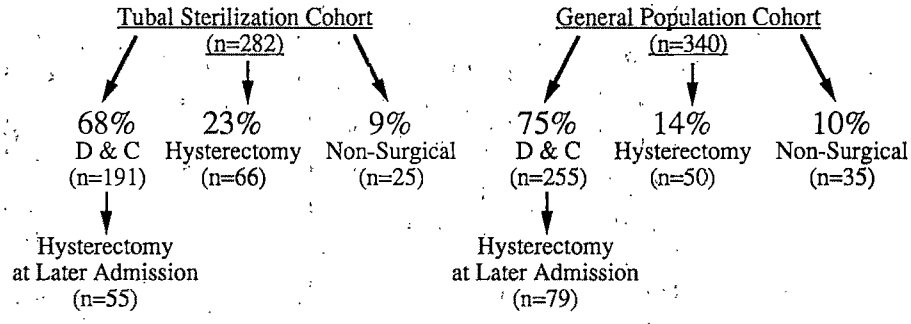


Fig. 1. Comparison of hospital management of menstrual disorders in general population.

Table V. Multivariate spouses comparison model* (based on married women with tubal sterilization compared with nonsterilized women with vasectomized spouse) to evaluate the risk of hospitalization for menstrual disorders after adjustment for potential confounding variables

Variable	*Slope (B)*	*Standard error of slope*	*Relative risk*	*95% confidence interval*	*Significance†*
History of menstrual disorder					$p < 0.01$
No	0.0	—	1.0	—	
Yes	0.91	0.30	2.5	1.4, 4.5	
Gravidity‡	0.048	0.087	1.0	0.88, 1.2	$p > 0.30$
Prior contraceptive method					$p > 0.20$
None	0.0	—	1.0	—	
Oral contraceptives	0.42	0.37	1.5	0.74, 3.2	
Intrauterine contraceptive device	−0.28	0.56	0.75	0.25, 2.2	
Other	−0.056	0.41	0.95	0.42, 2.1	
Tubal sterilization					$p < 0.01$
No	0.0	—	1.0	—	
Yes	0.71	0.26	2.0	1.2, 3.4	

*This was an unmatched (unconditional) logistic regression model based on analysis of 105 women who were hospitalized for menstrual disorders and 205 women who were not. Age and duration of Group Health Cooperative membership were also entered as variables, but for concisensss the results are not listed. (See text for explanation of sampling procedures.)

†p Value for exposure tests difference in risk across categories (based on deviance test).

‡Coded as continuous variable (i.e., 0, 1, 2, 3, etc.).

with unipolar sterilization compared with other sterilization methods. Thus on the basis of our data an increase in menstrual disturbance related to interference with the uterotubal circulation seems unlikely.

Our data were insufficient to determine patients' underlying likelihood (or preference) for elective surgery. Preference for elective gynecologic surgical treatment could prompt elective surgery (by tubal sterilization) for contraception and elective surgery (by curettage or hysterectomy) for treatment of menstrual disorders. Similarly, other women may have a preference for nonsurgical contraception and nonsurgical treatment of menstrual disorders. Because couples choosing permanent contraception by vasectomy (rather than by female sterilization) are relatively adverse to elective gynecologic surgery, preference for nonsurgical treatment would probably have had the greatest effect on the results from the spouses' comparison. In our results, the adjusted risk of hospitalization for menstrual disturbance after sterilization was only slightly greater in the spouses' comparison relative to the population comparison. Shain et al.[13] found that women whose husbands have undergone vasectomy are more likely than married women with tubal sterilization to be especially fearful of reproductive surgery.

Scott[11] suggested that because future pregnancy is not a consideration after tubal sterilization, patients and physicians are more likely to choose elective hysterectomy as treatment for gynecologic problems such as menstrual disorders. In our study, among women with their first hospitalization for menstrual disorders, 23% in the sterilized cohort and 24% in the sterilized spouses cohort were submitted directly to hysterectomy, versus 14% from the general population cohort and 16% from the vasectomy spouse cohort (Figs. 1 and 2). This practice difference is consistent with Scott[11] and suggests that patients' and physicians' views regarding the reduced need to preserve reproductive potential may have contributed to the increase in hospitalization for menstrual disorders we observed after tubal sterilization.

At least two sources of bias may have affected our

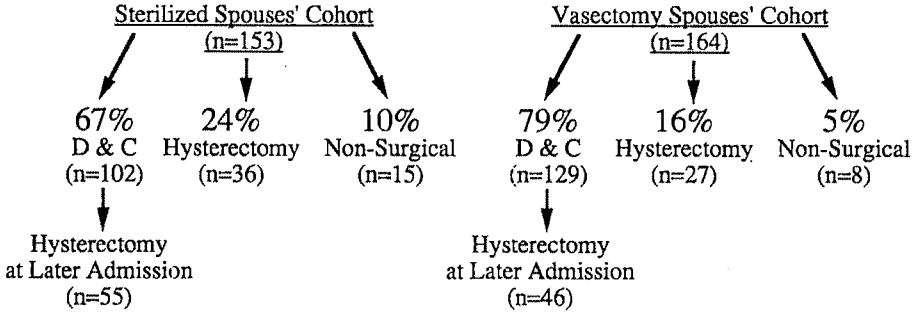


Fig. 2. Comparison of hospital management of menstrual disorders in the sterilized spouses.

results. First, because our data did not include the medical records of patients before enrollment in Group Health Cooperative, we probably included some women with prior tubal sterilizations in the nonsterilized cohorts. This bias (if present) would reduce the magnitude of our estimate of the risk of menstrual disorders. Alternatively, if women with prior hysterectomy were included in the nonsterilized cohorts, the risk of menstrual disorder would have been overestimated by our study. Second, because ours was not a randomized trial, we cannot exclude the possibility that residual confounding affected our results.

Most studies that compared menstrual events of sterilized and nonsterilized women suggest an increased risk of menstrual disorders after tubal sterilization.[14-17] These prior studies did not use hospitalization as an outcome, and they are troubled by limited follow-up time and the possibility of reporting bias because they are based on data from self-reported questionnaires.[14-17] In one study[17] that found an association between tubal sterilization and menstrual disorders, there was no significant difference between the two groups in self-reported physician visits for menstrual problems, and the increase occurred chiefly among women with preexisting menstrual disorders. Women with prior hospitalization for menstrual disorders were excluded from our study.

Because the outcome of our study was hospitalization, it is doubtful that our data can be applied to lesser changes in menstruation that do not warrant hospitalization. Nonetheless, studies[18, 19] of reported menstrual flow before and after sterilization are consistent with our findings that do not suggest a definite effect of the sterilization procedure itself. In the single study[20] that actually measured menstrual blood, no difference was found before and after tubal sterilization.

In summary, a direct risk of severe menstrual disorders resulting from tubal sterilization has little substantial support in our data. Increased tubal destruction associated with unipolar sterilization was not associated with increased risk for hospitalization for menstrual disorders. The increase in risk we observed was subject to bias resulting from women's underlying preference for, or against, elective surgical care. Direct submission to hysterectomy (rather than curettage or medical treatment) for a larger proportion of women hospitalized for menstrual disorders after tubal sterilization suggested physician (or patient) preference for hospitalization and hysterectomy (vs nonsurgical treatment) when maintenance of reproductive potential was no longer an issue in the treatment of menstrual disorders. We conclude that, although sterilized women are hospitalized more frequently for menstrual disorders, this finding may be explained by factors other than a biologic effect of the sterilization procedure itself.

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Discussion

Dr. Robert Israel, Los Angeles, California. In 1951 Williams et al.[1] from Vanderbilt University published the first article on the possible relationship between tubal sterilization and subsequent abnormal uterine bleeding. They reported on the incidence of abnormal uterine bleeding in 200 women who had undergone prior tubal ligation compared with a group of nonsterilized women. Although the incidence of subsequent menorrhagia in the sterilized patients was similar to that in a randomly selected control group of hospitalized gynecology patients, the authors concluded that somehow women who have had a tubal sterilization later have a higher percentage of abnormal bleeding than would be expected "in the general female population." They concluded that, "in view of the very low mortality and morbidity resulting from simple hysterectomy in the absence of distortion of pelvic anatomy," hysterectomy rather than tubal sterilization should be considered "in those individuals with chronic or progressive diseases warranting destruction of their reproductive function. Procedures short of hysterectomy preserve only the menstrual function of the uterus and leave an organ that is, as far as is known, not only worthless but potentially dangerous." The study was retrospective, lacked appropriate matched controls, and ignored any preexisting menstrual disturbances in the sterilized women but did use life-table analysis. As you have just heard from Dr. Shy and the Seattle group, in the intervening 40 years we have become more statistically correct and much more circumspect in our conclusions.

Irregular cycles, menometrorrhagia, and dysmenorrhea occurring after tubal sterilization have been espoused as the leading representatives of the dreaded "posttubal syndrome." However, in spite of the 40 years from Williams to Shy, the question of whether tubal sterilization increases the risk of subsequent menstrual disturbances remains unsolved. The failure to definitively answer this question revolves around many confounding factors: (1) Obtaining an accurate, long-term, prior menstrual blood flow and cycle regularity history from patients is subject to significant recall bias; (2) discontinuing oral contraceptives coincident with the surgery may be the culprit in many instances rather than the operation itself; (3) many women will experience menstrual disturbances over time irrespective of whether a tubal sterilization was performed. It is becoming apparent, from the myriad number of studies that have attempted to look at the "posttubal syndrome," that if menstrual disturbances occur after tubal sterilization, they may take quite a while to develop and are more likely to occur in women who had abnormal presterilization menstrual histories. In addition to long-term, post-tubal-sterilization follow-up studies that include office or clinic visits for abnormal uterine bleeding and hospital admissions, there is a definite need for studies in which women have both presterilization and poststerilization hormonal determinations carried out to see if disturbances occur that may affect subsequent ovulatory function.

As with the Seattle group's earlier article,[2] this effort is well done, well written, statistically overwhelming, and provocatively appealing in its conclusions. First, women who elect tubal sterilization would be more likely to choose surgical treatment rather than medical therapy for any subsequent menstrual disorders. Second, these previously sterilized women, and their doctors, are more inclined to select hysterectomy for definitive relief.

Finally, I would like to compliment the authors for their honesty in clearly delineating the key deficiencies in the study. The failure of the automated data base to contain factors such as gravidity and prior contraception did eliminate an important area, the assessment of confounding variables. As they noted, with the nonrandomized nature of the study they could not exclude the possibility that the absent residual confounders adversely affected the overall results. In the 40 years that have passed since the Vanderbilt article, it is obvious that our research has become more sophisticated and our statistical designs more discriminating. With the passage of time and the improvement of scientific methods, these trends might have been expected. What might not have been anticipated is what Shy et al. have demonstrated so beautifully today—that even a negative study can be presented with unassuming integrity without resorting to the hyperbolic conclusions of the 1951 article.

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DR. DUNCAN R. NEILSON, Portland, Oregon. Dr. Shy, oral contraceptives reduce the frequency of menstrual abnormalities. How many patients in the control group were taking oral contraceptives? Did you compare the poststerilization patients with controls not using oral contraceptives?

DR. SHY (Closing). Dr. Neilson, contraceptive histories are complex. We could have controlled for the contraceptive method in use just before the sterilization procedure. We chose, however, to control for the contraceptive method in use 6 months before sterilization because that seemed more relevant. In the analysis, when we stratified for contraceptive method used before sterilization, there was no modification in the conclusions.

Early amniocentesis: Outcome, risks, and technical problems at ≤12.8 weeks

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OBJECTIVE: It is the purpose of this report to evaluate our experience with amniocentesis at ≤12 weeks' gestation.

STUDY DESIGN: Medical records of 936 patients at ≤12.8 weeks' gestation undergoing genetic amniocentesis between Oct. 1, 1986, and June 30, 1990, were evaluated for gestational age, indication, frequency of needle insertion, amniocentesis complications, and pregnancy outcome.

RESULTS: There were seven miscarriages within 2 weeks of amniocentesis (0.7%), 21 miscarriages before 28 weeks (2.2%), and four stillbirths or neonatal deaths (0.4%), resulting in a total postprocedural loss rate of 3.4%. There were 26 chromosomally abnormal fetuses (2.8%). The spontaneous abortion rate in ultrasonographically normal pregnancies at <14 weeks, not undergoing amniocentesis, has been estimated at 2.1% to 3.2%.

CONCLUSION: Amniocentesis at 12 weeks is a viable option for patients desiring earlier prenatal genetic diagnostic information. (Am J Obstet Gynecol 1992; 166:1707-11.)

Key words: Early amniocentesis, miscarriage, amniotic fluid leakage

The prenatal diagnosis of chromosomal and biochemical abnormalities and neural tube defects is now a routine part of obstetric management. We can now offer patients a spectrum of diagnostic procedures commencing at 8 weeks' gestation including chorionic villus sampling, early amniocentesis, and standard amniocentesis.

Our first report concerning early amniocentesis appeared in 1987[1] and outlined our outcome, risk, and technical problem experience. This was followed by a second report, presented in 1989 and published in 1990,[2] that included continuous ultrasonographic guidance as a routine procedure for early amniocentesis. This report was criticized for a paucity of procedures performed at ≤12 weeks, with a recommendation for the publication of such data in the future to address that deficiency.

It is the purpose of this report to evaluate our experience with amniocentesis at ≤12 weeks.

Methods

The University of California, Davis prenatal diagnosis program has been previously described.[1-3] Since the inception of the program in 1976, >20,000 amniocentesis procedures have been performed, with more than 3000 of them before 15 weeks' gestation. The majority of these procedures have been performed by F.W.H. During the period of time covered by this report (Oct. 1, 1986, to June 30, 1990) 8165 amniocentesis procedures were performed. Each year 18% to 26% of the total was performed because of abnormalities of maternal serum α-fetoprotein levels detected as part of the California Maternal Serum Alpha-Fetoprotein Screening Program.

The data were evaluated for amniocentesis indication, gestational age at the time of amniocentesis, frequency of needle insertion, amniocentesis complications, and pregnancy outcome. For comparison with previously published studies, pregnancy loss data were divided into the following categories: ≤2 weeks after amniocentesis, ≤28 weeks' gestation (miscarriage); ≥28 weeks' gestation (stillbirth), and neonatal death (within 1 week of birth). Loss data were further evaluated as to possible or probable causal relationships to amniocentesis.

The amniocentesis technique used in our program has been previously described.[1-3] Continuous ultrasonographic monitoring has been routinely used since Oct. 1, 1986, to guide needle placement and monitor fetal movement during amniocentesis. We use an ATL Ultramark 4 ultrasonography machine (Advanced Technology Laboratories, Bothell, Wash.) for this purpose. A 22-gauge spinal needle is routinely used, with removal of approximately 1 ml of amniotic fluid (maxi-

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6/6/37160

Table I. Indications for amniocentesis

Indication	*No. of patients*	*%*
Maternal age	806	91.7
Family history or prior child with chromosome abnormality	36	4.1
Maternal anxiety	24	2.7
Family history or prior child with neural tube defect	5	0.6
Prior child with multiple congenital abnormalities	2	0.2
Fetal abnormality on ultrasonography	2	0.2
Other	2	0.2
TOTAL	879	100.0

Excludes 57 patients from Oct. 1, 1986, to Sept. 30, 1987.

Table II. Gestational age at time of amniocentesis on basis of biparietal diameter measurements

Biparietal diameter (cm)	*Gestational age (wk)**	*No. of patients*	*%*
2.2	12.8	242	27.5
2.1	12.5	238	27.1
2.0	12.2	172	19.6
1.9	11.9	88	10.0
1.8	11.6	75	8.5
1.7	11.3	38	4.3
1.6	11.0	13	1.5
1.5	10.7	9	1.0
1.4	10.4	4	0.5

Excludes 57 patients from Oct. 1, 1986, to Sept. 30, 1987.
*Weeks since last menstrual period ± 1 week.

mum 18 ml) for each week of gestational age, which is based on the measurement of fetal biparietal diameter.

Amniotic fluid is cultured with the in situ technique of Peakman.* Fifteen clones and five karyotypes were usually examined, analyzed, and reported. The experience of our cytogenetic laboratory in the culture of amniotic fluid from 10 to 14-week pregnancies has been reported.[4] The mean culture time is 8.8 days (SD 1.5 days).

Results

The demographic data of the study population were not different when compared with those published in our previous studies.[1-3] The indications for amniocentesis are seen in Table I.

A total of 8165 amniocenteses was performed during the study period, including those performed because of abnormal maternal serum α-fetoprotein levels. Only patients with viable singleton pregnancies were included in this study. All preamniocentesis fetal deaths and multiple gestations were excluded from this study. There were 936 amniocenteses (performed at ≤12.8 weeks since the last menstrual period) that met these criteria, and they represent 11.5% (936/8165) of the total. The patient distribution by gestational age (as determined by biparietal diameter) is demonstrated in Table II. With the exception of three patients in 1990, all amniocenteses were performed by the same operator.

*David Peakman and Associates Reproductive Genetics Center, P.C., Denver, Colo.

Table III. Frequency of needle insertion

No. of insertions	*Frequency*	*%*
1	852	96.9
2	26	3.0
3	1	0.1

Excludes 57 patients from Oct. 1, 1986, to Sept. 30, 1987.

Table IV. Complications within 2 weeks of early amniocentesis

Complication	*No. of patients*	*%*
None	914	97.6
Fluid leakage	10	1.1
Miscarriage <2 wk	7	0.7
Bleeding	4	0.4
Cramping	1	0.1

The frequency of needle insertion is demonstrated in Table III. A total of 96.9% (852/879) were successfully performed with a single needle insertion. In one patient a retap was done because of inadequate cell growth, and subsequently the pregnancy was lost.

The volume of amniotic fluid removed varied from 10 to 18 ml. We have previously shown that the volume of amniotic fluid removed does not appear to influence an adverse pregnancy outcome.[3]

Table IV shows the number of patients who had complications within 2 weeks of amniocentesis. Of all the patients 97.6% (914/936) did not experience problems. Seven patients or 0.7% (7/936) had an unanticipated pregnancy loss within 2 weeks of amniocentesis.

Ten patients (1.1% of the total) had amniotic fluid leakage of several days' duration after amniocentesis. The pregnancy outcomes for the 10 patients with amniotic fluid leakage are seen in Table V. Four patients representing 40% (4/10) of the total number experiencing leakage after amniocentesis lost the pregnancy, and six patients or 60% (6/10) experienced term deliveries.

Table V. Pregnancy outcome in patients with amniotic fluid leakage

Case No.	*Gestational age (wk)*	*Complications before amniocentesis*	*Pregnancy outcome*
1	12.5	None	Normal term vaginal delivery
2	12.5	None	Normal term vaginal delivery
3	12.5	Severe spotting and cramping	Fetal death ≤2 wk
4	12.8	None	Fetal death ≤2 wk
5	12.2	None	Normal term vaginal delivery
6	12.2	None	Fetal death ≤2 wk
7	12.2	None	Normal term vaginal delivery
8	12.5	None	Normal term vaginal delivery
9	12.5	None	Fetal death ≤2 wk
10	11.8	None	Normal term vaginal delivery

Pregnancy outcome for all patients included in this study is listed in Table VI. A total of 92.4% (865/936) of patients were delivered of a normal child. No patients were lost to follow-up.

Six patients (0.6%) were seen with a variety of abnormalities, such as cleft lip or palate, club foot, and hydrocephalus, that were not detected prenatally. These were of insufficient frequency to rate placement in individual categories. Unlike our previously published studies,[1-3] no patients with fetal death before amniocentesis were included in this study.

Twenty-six patients (26/936) or 2.8% had fetuses that were structurally or chormosomally abnormal and chose to terminate their pregnancies.

Thirty-two patients representing 3.4% (32/936) of the total experienced losses at some point after the amniocentesis. Seven (0.7%) of the total (7/936) experienced a loss within 2 weeks of amniocentesis. Twenty-one or 2.2% (21/936) experienced a loss between 2 and 28 weeks after amniocentesis. There were four or 0.6% (4/936) stillbirths or neonatal deaths.

Four of the seven patients with losses within 2 weeks of amniocentesis experienced amniotic fluid leakage. One of these patients had two prior spontaneous abortions and severe cramping and bleeding for 2 weeks before the amniocentesis. Retrospectively, amniocentesis probably should have been delayed in this patient. No other preamniocentesis factors were identified to account for these losses.

Evaluation of the losses occurring >2 weeks and <28 weeks (21 patients) revealed that 14 patients had one or more normal visits to their referring physicians before documentation of the loss. Other complicating factors such as omphalocele, diabetes, preamniocentesis bleeding, chromosomal mosaicism, or umbilical cord accident could also be identified in these patients. Seven patients did not have any plausible factor identified to indicate that the loss was not procedure related.

The overall loss rate of desired pregnancies in this study was 6.2% (58/936). This percentage represents a summation of those losses occurring after ammniocentesis (n = 28), stillbirth or neonatal deaths (n = 4), and elective termination of abnormal pregnancies (n = 26).

Table VI. Pregnancy outcome for 936 patients undergoing amniocentesis at ≤12.8 weeks

Outcome	*No. of patients*	*%*
Normal fetus	865	92.4
Fetus abnormal—pregnancy terminated	26	2.8
Miscarriage ≤28 weeks' gestation	21	2.2
Premature delivery, <35 weeks' gestation	4	0.4
Miscarriage at ≤2 weeks after amniocentesis	7	0.7
Stillbirth or neonatal death	4	0.4
Abnormal child not detected prenatally	6	0.6
Small-for-gestational-age infant	1	0.1
Other	2	0.2

Comment

A variety of prenatal diagnostic procedures, including traditional amniocentesis (15 to 18 weeks), earlier amniocentesis (<15 weeks), and chorionic villus sampling (8 to 12 weeks), are now available to obstetric patients. Numerous reports have been generated with regard to the safety and hazards of these procedures.[1-3, 5-9] Fetal loss rates for amniocentesis have ranged from a low of 0.3%[9] to 2.8%.[5] A combined loss rate for desired pregnancy (spontaneous and missed abortion, termination of abnormal pregnancy, stillbirth, and neonatal deaths) of 7.2% has been reported for chorionic villus sampling.[10] Their amniocentesis

control group had a 5.7% loss rate. Adjustment of data led to the conclusion that a 0.8% difference in loss rate existed for the two groups and favored amniocentesis.

Our center has previously published a 4.4% figure for loss of desired pregnancies when amniocentesis was performed at <15 weeks' gestation.[3] That study was criticized for being heavily weighted with patients who were at 13 and 14 weeks' gestation. A comparable figure for this study is 6.2%.

There are now other studies in the literature evaluating early amniocentesis. Elejalde et al.[11] reported the loss of four of 77 (5.2%) fetuses <13 weeks within 2 weeks of amniocentesis. Nevin et al.[12] in a small series lost no fetuses between 9 and 12 weeks ($N = 25$). Benacerraf et al.[13] reported no losses in 100 patients at <15 weeks after a 5-week follow-up period.

The establishment of a procedural risk factor for comparison with other studies and for patient counseling is important yet difficult. All prenatal diagnostic procedures are performed against a certain background of spontaneous abortion. The earlier a procedure is performed, the higher is that loss rate and the longer is the opportunity for subsequent spontaneous loss. Lack of physician experience, poor reproductive history, and increasing patient age have all been shown to be possible factors in increased loss rates.

It has generally been accepted that 15% to 20% of recognized pregnancies result in spontaneous abortion before 20 weeks' gestation. Liu et al.[14] reported an overall 2.7% spontaneous abortion rate in pregnancies found to be ultrasonographically normal before 14 weeks, with 1.5% occurring before 16 weeks' gestation. A higher loss rate was seen in primigravid patients (1.8%) and threatened abortion patients (38%). Gilmore and McNay[15] and Wilson et al.[16] reported similar findings with an overall loss rate of 2.1% and 2.13%, respectively. The 1976 National Institute of Child Health and Human Development study demonstrated a 3.2% spontaneous abortion loss rate in their control group of women matched for race, gravidity, and income.[5]

Our overall loss rate of ultrasonographically normal pregnancies was 3.4% (32/936). If one accepts a background loss rate of 2.1%, we are left with a difference of 1.3%. At least 2% ($n = 19$) of the losses (four stillbirths or neonatal deaths, 14 losses >2 weeks after the procedure, and one before 2 weeks) had one or more documented normal visits with their referring obstetrician or another possible cause of the loss. Evaluation of these data would suggest that in experienced hands amniocentesis at ≤12.8 weeks may have only a slightly, if any, increased risk of loss when compared with standard amniocentesis.

Technically, continuous ultrasonographically guided early amniocentesis is easy to perform. The experienced physician will reschedule those patients who have amnion-chorion separation and seemingly no available area of amnion attachment to the uterine wall, because they are more likely to subsequently have a leakage of amniotic fluid. Obesity, recent bleeding and cramping, a retroverted uterus, bladder or bowel superimposition over the uterus, and decreased fluid volume are also reasons to consider rescheduling amniocentesis in some patients.

In summary, it would appear that amniocentesis at 12 weeks is a viable option for patients desiring earlier prenatal genetic diagnostic information.

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Discussion

Dr. Gerald M. Shefren, Palo Alto, California. In 1989 Dr. Hanson presented to this society a series of ultrasonographically guided early amniocenteses in singleton pregnancies. In that series of 308 patients only 70 were listed as being <13 weeks' gestation. In the series presented the authors have retrospectively reviewed 936 patients of <13 weeks' gestation among 8165 amniocentesis procedures performed from 1986 to 1990. Dr. Hanson is to be congratulated, for this represents by far the largest series reported of first-trimester amniocenteses.

Comparison of these two reports shows a lower complication rate in the larger series reported here and only a slightly higher rate than in the midtrimester group when corrected for spontaneous background loss. Miscarriage within 2 weeks of the procedure dropped from 1.7% to 0.7%. Total procedural losses decreased from 4.7% to 3.4%. Success with first needle insertion increased from 88.5% to 97%. It seems clear that even experienced operators with midtrimester amniocenteses will have higher complication rates at first and lower rates as they gain experience.

One interesting difference was seen in the outcomes of patients experiencing fluid loss after the procedure. In the first series only one of eight had a fetal death. In this latest series four of 10, or 40%, of those with leakage of amnionic fluid experienced a fetal death within 2 weeks. This comparison appears to carry a much graver prognosis in first-trimester amniocenteses than it does in the midtrimester.

If the safety and accuracy of diagnosis can be assured with early amniocentesis, it would clearly invite obstetricians and their patients to consider it in place of chorionic villus sampling. At this time there are >60,000 patients who have undergone chorionic villus sampling worldwide. There have been several international multicenter, prospective, randomized studies published. Many, like the Medical Research Council report, compared chorionic villus sampling with midtrimester amniocentesis. Most studies showed some increased risk of chorionic villus sampling over midtrimester amniocenteses of 1% to 2% in total pregnancy losses. However, accuracy in detecting mosaicism, false-positive and false-negative rates, and the ability to measure α-fetoprotein and acetyl-cholinesterase clearly favor amniocentesis.

Comparing the results of the prospective randomized studies with the results presented by Dr. Hanson would be difficult, especially as they relate to patient selection. It seems possible that the patients in this report may have been selected as low risk by the referring obstetricians or the patient herself simply because it was a nonstandard approach.

I support the suggestion of other authors that until early amniocentesis is subjected to a large, randomized, prospective study the routine referral of patients for early amniocentesis seems premature. With that caveat in mind, the data in this series seem to support Dr. Hanson's conclusion that early amniocentesis appears to be a viable option for patients seeking first-trimester genetic diagnosis.

I have several questions. Do you have any data on the percentage of patients who would need to be rescheduled for technical reasons if all amniocenteses were attempted at <13 weeks' gestation? Because the mean amount of amniotic fluid is about 65 ml when most of the patients in your series underwent the procedure and if one removes 1 ml per week of gestation, some patients would have an acute amniotic fluid loss of 20%. Have you rescanned any of these patients over an extended time after the tap to see how long it may take to reaccumulate the lost fluid, if in fact it can be quantified at all?

Dr. Bruce B. Rolf, Los Angeles, California. What about the six abnormalities you missed?

Dr. Hanson (Closing). Dr. Shefren, because of our wide catchment area, we have some patients who traveled long distances and for them rescheduling is a real problem. Our rescheduling rate is <2% because our staff thoroughly screen the patients. If we know that the patient has a retroverted uterus or weighs >250 pounds, we schedule the procedure later in pregnancy.

In regard to amniotic volume, we have an unspoken agreement with the sonographers that if they see any change in amniotic cavity size, they tell me and I stop.

Dr. Rolf, I left out all discussion of undiagnosed abnormalities such as hydrocephalus and cleft lip and palate because we have discussed them in other articles. You would not expect to detect hydrocephalus before 18 to 20 weeks and cleft lip and palate are difficult to detect at 12 weeks. To the best of my knowledge, no abnormality that should have been picked up was missed by ultrasonography associated with any of the early amniocentesis procedures I have reported.

TRANSACTIONS OF THE FIFTY-NINTH ANNUAL MEETING OF THE CENTRAL ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS

Suffer the little children

Presidential address

James P. Youngblood, MD
Kansas City, Missouri

As I set about planning this address, I reviewed many of the recent addresses given by our esteemed past presidents. They are generally in one of three categories: historic, scientific, or philosophic. They have nearly always been of a personal nature, to a greater or lesser degree, revealing something of the inner character of the speaker. Dr. Russell Malinak's address last year was especially stirring and memorable. I recall one of our members asking me immediately after the talk whether I felt that I could top it this year. Of course, I won't be able to, and I won't even try. But I hope at the end I will have given you some food for thought, and perhaps you'll also know a little more about me.

My talk is more of the philosophic type, and I think it will reveal a little of how I think and what has become very important to me over the years.

The title of this address is "Suffer the Little Children. . . ." What do I mean by that? I think that most of you are aware that this is from the New Testament, the Gospel according to Mark, chapter 10, verse 14. "Suffer the little children to come unto me and forbid them not, for such is the Kingdom of God." After preaching all day, Jesus is tired and is resting and the little children in the area have come for his words and his blessing, and his disciples are trying to keep them from him so that he may rest. In this case, Jesus uses the term *suffer* to mean *permit.* He indicates that the children have a priority higher than any need that he may have for rest. Indeed, he indicates their helplessness and innocence in the words "for such is the Kingdom of God."

I have used the word *suffer* in a double context in this address, because the children in our world and in our country do suffer. And, of course, they are innocent. By now you are certainly aware that we have a problem with infant mortality in this country and that it is a national embarrassment. The richest nation on earth, now the only super power, with the most advanced medical technology, which spends 11%[1] of its gross national product on health care, ranks nineteenth to twenty-second[2] in the world in perinatal mortality. The most recent figures indicate that the overall perinatal mortality in the United States in 1988 was 13.7.[3] This means that per 1000 live births 13.7 babies die either before or during birth or within the first 28 days of life after birth. In 1990 in the county in which I live the perinatal mortality rate was 13.0[3] overall and 21.4 for blacks.[4] This is pathetic.

So here we go again—another talk about perinatal mortality and America-bashing because we're doing such a lousy job. I'm sorry, but the problem is severe and must be kept in the public consciousness. But we're not all that bad. We are doing some things to turn the problem around and we are making progress. But more must be done.

I know that you are concerned that this talk will be a reiteration of Dr. Malinak's eloquent address last year.[5] You must forgive me for having chosen the same topic, but realize that this has been my main area of concentration for the past 7 years and I have thought long and deeply about this problem. I may cover some of the same ground, but I think you'll see that we have somewhat divergent views in the solution of the problem and therefore I feel it merits exploration. In the long run I do not wish you to consider this a rebuttal of Dr. Malinak's address but rather an exploration and possible expansion.

While in the private practice of obstetrics and gynecology for 20 years, I was aware that there was a perinatal mortality problem. But it was in the inner city, and although I frequently attended clinics in the inner city, I was not completely aware of how extensive and pervasive the problem was. It wasn't that I didn't care, it was that I was just unable to interact in a meaningful way.

Seven years ago, I undertook the chairmanship of an obstetrics and gynecology department in an inner-city public hospital. I was unprepared for what awaited.

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6/6/37130

The indigent and working-poor population had been increasing—Nutrition was poor, disease of all kinds was rampant, and medical complications of pregnancy were numerous. Resources of all kinds were inadequate to meet these demands. The low-birth-weight rate at our hospital was 15% as compared with a national average of 6.9%. Then came Medicaid expansion and we were able to increase staffing and more actively apply our interventions, especially for so-called high-risk pregnancies. But, in spite of all our efforts, the low-birth-weight rate remained at 15%. Consequently, we developed the premise that risk assessment for pregnancy as it was done in most institutions was invalid in our institution. It was our impression that the patients we called routine patients were, indeed, all at high risk. With the support of a 2-year grant from The March of Dimes, we set about proving this premise. We studied 300 routine patients in the study group against 300 similar routine patients in the control group. The study group was intensively educated, with emphasis on things such as awareness of premature contractions, health and diet, and education regarding smoking, and drug and alcohol abuse. Socioeconomic problems were more intensely addressed. In other words the study group of routine patients was treated as high risk. Amazingly, the low-birth-weight rate in the routine study group, who had intensive prenatal care and education, was 7.3%, whereas the rate in the so-called routine control group was 14.8%. Therefore a decrease of 7.5% in the low-birth-weight rate occurred.[6] Although it was a small study, the numbers were compelling and I was able to convince our hospital director to increase our staffing of nurse practitioners, nurses, health care educators, nutritionists, and social workers in our obstetric clinic so that we could intensify our care across the board for all of our patients. In January 1991 we instituted this plan and look forward to a positive report on the results in the near future.

But intensity of prenatal care is not the only answer. Prenatal care is carried on within the institutional walls or doctors' offices and does not address the problems of access, transportation, economics, and the social and psychologic difficulties of the patient population. Nor does it address the role of poverty—poverty rooted in habits and character traits—"behavioral poverty" George Will called it in his recent syndicated column of Sept. 29, 1991.[7] Nor does it address the teenage pregnancy problem and particularly the cultural sensitivity of our various racial and ethnic subgroups. Yet, in addition, we must also stress responsible parenting. Too long have we witnessed teenagers spring and bearing three or four children before age 20. Not only safe sex but responsible sex must be encouraged as in the American College of Obstetricians and Gynecologists' "I Intend" program. Finally, we must address the role of government, industry, and the medical profession itself.

In this country we tend to excuse our poor performance with perinatal mortality relative to European countries by stating that we're comparing apples and oranges. Societies in Scandinavia, Germany, France, and England are all homogeneous, whereas in the United States we have a much more heterogeneous society composed of various cultures, ethnic backgrounds, and races. We somehow rationalize that this prevents adequate prenatal care and universal access to such care. The major difference is that in all of the European countries and in Japan universal access to prenatal care is not only available but mandated by the governments of these countries.

In the 1989 report of the United States Public Health Service expert panel on "Content of prenatal care," many factors that may affect perinatal mortality were cataloged.[8] Without going into great detail, these can be subdivided into medical disorders before pregnancy, such as hypertension and diabetes, and specific pregnancy conditions or hazards, such as preeclampsia and infection. There also are psychosocial risk factors, including being a single parent, having a limited formal education, and living in poverty. This leads to the next item of socioeconomic status, which includes occupation, education level, housing, income, marital status, and nutritional resources. Seldom considered but extremely important are psychologic factors such as limited maternal support networks and increased levels of stress because of pregnancy, emotional disorders, and, of course, pregnancy ambivalence. Increasingly, adverse health behaviors have contributed to perinatal mortality and these include, as we well know, drug and alcohol abuse and smoking.

I submit that we can address the medical problems associated with pregnancy and are doing so within our institutions when we have early registration into the care system by our mothers. It is the socioeconomic and psychosocial factors and the cultural and ethnic factors that prevent us from applying our medical technology to the fullest with the expectation of good outcome. We must get out of the institutions and into the community to address these problems. We must begin education and provide resources to attract women not only to early prenatal care but even to preconception care. It is imperative that we form a partnership of influencing factors, government, industry, and the health care community and women themselves who can work together to address this problem of high perinatal mortality. Let's take a look at what these entities can do.

What can government do? In 1986 Congress formed the National Commission to Prevent Infant Mortality. Its charge was to create a national strategic plan to reduce infant mortality and morbidity in the United

States. The Commission includes some members of Congress, the Secretary of Health and Human Services, and the Comptroller General of the United States. One of the unique programs this National Commission has developed is the concept of home visiting, especially by resource mothers. Resource mothers are women selected from the community who have evident helping skills and who have been successful mothers. They receive training in health, social services, and counseling to enable them to help disadvantaged young mothers, usually teenagers, get early prenatal care and reduce or eliminate unhealthy behaviors, such as smoking, drinking, and other substance abuse. In addition, these resource mothers help prepare the patients for labor and delivery and the needs of a newborn. These women are generally neighbors and friends of the young women they visit and therefore are culturally sensitive and aware of not only their health needs but also their psychosocial and economic needs. They also visit the mothers and babies after delivery and ensure the infants receive regular checkups and that the health of mother and baby is enhanced and ensured. Where these programs have been established, there has been a marked lowering of the low-birth-weight rate.[9] We are working now to promote this kind of activity in our own area and anticipate such activity catching on throughout the nation.

What can we expect from Industry? You should know that in all of the countries I've mentioned, in Europe and Japan, industry in some way or another subsidizes pregnant women. In France women are paid their normal salaries for 6 weeks before delivery and 6 months after delivery. Also, a doctor may prescribe additional compensated rest whenever indicated.[10] How can they afford to do this? The question should be, how can we in the United States afford not to subsidize our pregnant women? You may be surprised to know that a few enlightened companies in this country are already subsidizing pregnancy. Mr. Joseph Taylor,[11] plant manager of Sunbeam Industries, Coushatta, La., is one of the early leaders in this kind of support for pregnancy.

What can we expect of medicine? What can we as obstetricians-gynecologists do to reduce infant mortality in the United States? Do we need to change our whole health care system? I think not. As Dr. Malinak so eloquently expressed in his address last year,[5] we have the finest health care system in the world, bar none. Our problem is getting that health care to those who need it in a timely manner to prevent low-birth-weight infants and high perinatal mortality.

There are things we can do. In his presidential address, our Immediate Past President of The American College of Obstetricians and Gynecologists, Ezra Davidson, presented a strategy to reduce infant mortality. Briefly, his strategy is to mimic the maternal mortality review committees that we used so effectively in the 30s, 40s, 50s, and 60s to reduce maternal mortality to a number <1 in this country. Dr. Davidson feels that similar local review committees should be established and every perinatal death should be reviewed as to cause and preventability. He feels, and justifably so, that such intensive peer review will reveal numerous strategies to respond to locally identified factors.[11]

I would strongly urge that you not only be active members of such committees in your area but even spearhead the formation of such committees if they currently do not exist. Nearly all of us belong to local obstetrics and gynecology societies or city or county medical societies whose resources could be used for this effort.

And what should we expect of our nation's mothers to help reduce perinatal mortality in the United States? They must be educated as to the necessity of early prenatal care and even of preconceptional care. They must be encouraged to enter the health care system at an early age, in grade school if possible. They should register early for prenatal care because pregnancy may be a delicate condition in their particular case. They may need special care and special interventions to ensure a good outcome for themselves and their babies. Adverse health behavior should be curtailed or modified. Smoking, drinking, and illicit drug use should cease as proper nutrition is emphasized. Many workplace and pregnancy-related studies that have been done link working conditions to increased adverse outcomes in pregnancy. For example, a job requiring standing all day is much more likely to produce preterm labor than a job requiring only part-time, upright working conditions.[12] Pregnant women and their employers need to be aware of the implications of their workplace situation and adjust accordingly.

Finally, in my mind there is really only one comprehensive and satisfactory solution for reducing perinatal mortality in this country. Universal access to early prenatal care! All of the factors I've just mentioned should be addressed, but universal access is the absolute necessity. All of our medical and social interventions are useless unless they are accessible. We are all aware that 37 million people in this country have no health insurance. Naturally, most of these people are young and many of them are women in the reproductive age group. Medicaid is addressing the needs of the very poor but not the working poor. We must address the needs of all pregnant women. You know we have a peculiar mindset in this country that everyone must adhere to the "pay-as-you-go" format for health care regardless of economic situation.

Have you ever considered that there is a very large

segment of our population that has to jump through only one hoop to have health care provided? There is no asset testing, no one's bank account is examined, there are no qualifications save one—you must be 65. Of course, I'm talking about Medicare. I remember in 1965 I had just started in practice and was appalled that Congress enacted the Medicare Act, feeling it would be the downfall of medicine. You'll recall that organized medicine came screaming and kicking into the Medicare reality. Obviously, its not a perfect system and is rife with problems and even corruption, yet it continues to be one of the more humane things that our society has done for its elderly.

As a matter of fact, in 1987 combined Medicare and Medicaid health care for the aged and disabled came to 114 billion dollars, or 23% of the health care dollar, as opposed to only 6 billion, or 2.5%, provided for maternal health care.[13] Are we emphasizing the wrong age group? Triple bypass surgery in a 75-year-old person costs $50,000; prenatal care costs $500.

I would propose that government, industry (including the insurance industry), and the medical profession form a partnership to produce a maternity insurance plan that would be available for all. The only requirement to enter the health care system to receive prenatal care would be a positive pregnancy test. No other hoops need be jumped through because no extensive application forms need be reviewed and there would be no asset testing. Imagine the decrease in the bureaucracy now used to administer Medicaid. Any pregnant woman regardless of race, ethnic background, or social status would be eligible for this care.

But, given the problems obstetricians currently have with Medicaid, such as low and slow reimbursement and the interminable forms and red tape, why would they buy into this plan?

First, organized medicine must negotiate a realistic global fee for prenatal care and delivery. This fee must take into account that many of the new and previously uninsured will be at high risk, as I have already outlined.

Second, because a positive pregnancy test allows immediate and early access to care, any necessary documentation and paper work can be accomplished throughout the course of pregnancy, thus ensuring prompt payment on completion of care.

How would a newly pregnant woman have access to care? She would simply obtain proof of pregnancy from any recognized health care provider (e.g., hospital, clinic, or her own physician). She would immediately receive a voucher for prenatal care by her provider of choice.

What about the woman who desires more than average amenities? Because the vouchers would be equal in value, she would need to personally supplement the reimbursement required for these amenities. As you know, this same system is currently used in European countries.

What should we call this plan? How about "Maternacare"? Call it what you will, but whatever you call it, it is progress for humankind and maternal and infant welfare.

Perhaps I've made this too simplistic. Believe me, I know that problems will ensue. Institution of this kind of system will strain the resources of the prenatal health care system as we know it today. When we instituted our own new program as I previously outlined to you, so markedly increased was patient interest and awareness that early registration and frequency of visits increased to the point that we swamped the system. Our physicians, both resident and attending, were unable to handle the load, and we had to hire additional physician extenders, such as nurse practitioners and physician aides. But this is already going on in many large obstetric groups and health maintenance organizations. I have personal knowledge in our own area of private practice groups that have hired nutritionists, nurse practitioners, and even midwives to help them with the enhanced prenatal care that they wish to deliver. Obstetricians will need to become comfortable with the idea that they will need the help of other professional health care providers to maximize their efficacy in driving down perinatal mortality in this country.

As I was writing this article, I mentioned this concept to several friends and associates, not necessarily physicians, and, to a person, the first question they asked was, "Who would pay for it?" Well, obviously, you and I would pay one way or another, but we already pay for Medicare and Medicaid. We pay for national defense, and we pay for fire and police protection and wildlife conservation. And how about the savings and loan bailout—the last estimate I read 2 weeks ago was 160 billion dollars! What I am asking for is peanuts!

Why not pay for our single greatest resource, our newborn infants? And, would it be very expensive? Yes, but it certainly is expensive now. Consider that a premature infant of 2 pounds who stays in the hospital up to 3 months may incur medical costs of $50,000 to $100,000 and possibly a lifelong cost of $400,000 to $1,000,000 if neurologic deficits are incurred. Consider that there are 280,000 low-birth-weight infants born annually[5] and each of them incurs on average $10,000 in additional medical costs before they can leave the hospital. This comes to a total of $2.8 billion. Consider if we could lower the low-birth-weight rate by 1% in this country, the savings would be $400,000,000 annually. If we could get our preterm delivery rate down to that of France, which is slightly over 4%,[15] the savings

would be more than $1 billion. This savings plus what is currently spent by government sources, insurance premiums, and private pay would come close to paying for the entire cost of universal access to prenatal care. Whatever the cost, the time has come for this nation to get its priorities straight and one of them should be a reduction in this terrible infant mortality that we continue to have.

National mindsets can be overcome. Even global mindsets can be overcome.

Consider Semmelweiss' realization that it was the unclean obstetrician who was the cause of puerperal sepsis—childbed fever. It was 30 years before his simple recommendations for antisepsis were accepted, after he had been driven mad by his critics and detractors.

Sometimes the simplest ideas may have most profound effects!

Surely we can overcome this mindset that is keeping us from enjoying our most precious resource—the infants and children of our country.

Suffer the little children to come unto us—healthy. And on time! Suffer the little children to suffer no more!

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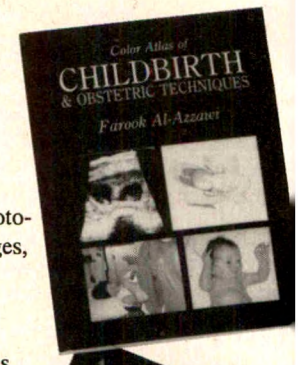
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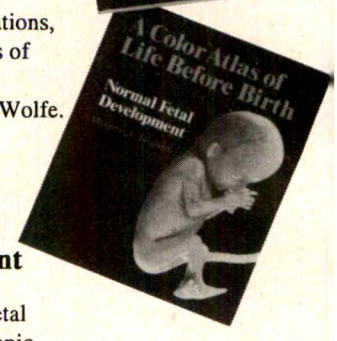
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FLR-813

Anatomic aspects of vaginal eversion after hysterectomy

John O.L. DeLancey, MD
Ann Arbor, Michigan

OBJECTIVE: Our aim was to understand how vaginal eversion after hysterectomy differs from other forms of prolapse.
STUDY DESIGN: The role of individual structures involved in vaginal support was studied by pelvic dissection of 61 cadavers. Serial cross sections from 13 additional cadavers were examined.
RESULTS: The upper third of the vagina (level I) is suspended from the pelvic walls by vertical fibers of the paracolpium, which is a continuation of the cardinal ligament. In the middle third of the vagina (level II) the paracolpium attaches the vagina laterally to the arcus tendineus and fascia of the levator ani muscles. The vagina's lower third fuses with the perineal membrane, levator ani muscles, and perineal body (level III). Dissection reveals that the paracolpium's vertical fibers in level I prevented prolapse of the vaginal apex and vaginal eversion.
CONCLUSIONS: The paracolpium in level I forms the critical factor that differentiates vaginal eversion from posthysterectomy cystocele-rectocele or enterocele in which the vaginal apex remains well suspended. (Am J Obstet Gynecol 1992;166:1717-28.)

Key words: Vaginal prolapse, vaginal eversion, hysterectomy

Vaginal eversion occurs after hysterectomy when the apex of the vagina descends below the introitus so that the vagina turns inside out. In recent years this problem has become a topic of considerable concern. In spite of our generally good success in treating vaginal eversion[1-3] we know little about its cause and prevention. Vaginal eversion is usually thought of as a problem caused by an enterocele that was not repaired at the time of vaginal hysterectomy for prolapse,[4] yet in half of the patients with posthysterectomy vaginal eversion the condition develops after an abdominal hysterectomy that was performed for reasons other than prolapse.[1, 4] In addition, this theory does not explain why some patients have an enterocele with a well-supported vaginal apex after hysterectomy whereas others have both an enterocele and an eversion of the vagina. These questions highlight the importance of knowing the specific anatomic defect that is responsible for vaginal eversion.

Many investigators have looked at the structural changes that are associated with uterine prolapse, cystocele, or rectocele, and these studies have been summarized elsewhere.[5] None of these reports have directly studied the anatomy of vaginal support as it relates to the problem of vaginal eversion after hysterectomy. It is possible to piece together some aspects of this anatomy by gathering the observations of several authors; however, this eclectic collection is characterized by controversy, conflict, and confusion[6, 7] and justifies the need for a specific examination of vaginal support after hysterectomy. The aim in studying vaginal support was to gain information that would help explain the anatomic difference between women with vaginal eversion and those with cystocele, rectocele, or enterocele after hysterectomy but in whom the apex of the vagina remained well supported.

From the Department of Obstetrics and Gynecology, University of Michigan Medical School.
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6/6/37132

Material and methods

Dissections. Several types of observation were used for this study to avoid the errors that can arise from artifacts present in any single technique of anatomic investigation. Vaginal support was examined in cadavers that had previously undergone hysterectomy, as well as in those with the uterus in place, so that we could study both the relationship of vaginal support to uterine support and the support of the vagina seen after hysterectomy (Table I). Dissections, which were performed on the pelvic viscera and the pelvic walls, focused on the connective tissue and muscles in the region bounded laterally by the bony pelvis and extending from the sacral promontory to the vulvar skin.

The importance of specific structures to vaginal support was studied in selected cadavers that were in an upright position, as if an individual were standing. Isolated portions of the vagina were forced toward the introitus for study of the structures that prevented prolapse or eversion from occurring. Special attention was paid to the tissues that resisted eversion of the vagina

Table I. Dissections

Cadaver preparation	*No. dissected*	*No. with previous hysterectomy*	*Age (yr)*
Fixed cadavers			
Embalmed cadavers	16	2	30-104
Immersed cadavers	3	1	26-56
Fresh (unfixed) cadavers	42	7	7 mo–87
TOTAL	61	10	

Table II. Whole-pelvis serial sections*

Age (yr)	*Parity*	*Orientation*	*Previous hysterectomy*
14	Nulliparous	Transverse	No
26	Multiparous	Coronal	Yes
28	Multiparous	Sagittal	No
33	Nulliparous	Transverse	No
64	Multiparous	Oblique	Yes

*All specimens fixed by immersion.

in cadavers with previous hysterectomy. Examination of cadavers that were in the upright position permitted the suspensory apparatus to be examined in the orientation in which it is called on to support the vagina (i.e., the standing position). This technique was found to be critical in the correct interpretation of the role that each part of the vaginal support system plays in holding the vagina in place.

The cadavers used in this study included both those fixed by the traditional injection technique and ones fixed by immersion. The immersion method was used to eliminate the distortion created by injection embalming. The normality of the spatial relationships in cadavers fixed by immersion was confirmed by comparing them with topographic studies of living individuals with normal support that are available in the literature.[8]

Serial cross sections. Both histologic and whole-pelvis cross sections were studied. The serial histologic sections were made available to me by Dr. Thomas Oelrich and have been described in previous works.[9, 10] They included 1500 sections from eight individuals 0 to 37 years old. These were used to confirm the histologic nature of the tissues identified in the whole-pelvis cross sections and to determine the specific direction of various fibers within the pelvis. Serial whole-pelvis cross sections were made from frozen cadavers fixed by immersion, as indicated in Table II. Sections were cut at intervals varying from 5 mm to 2 cm an stored in formalin.

Results

Overview. The bladder, uterus, vagina, and rectum are all attached to the pelvic walls by a network of connective tissue strands that are collectively called the endopelvic fasciae. Although the connective tissue of the endopelvic fascia is one continuous unit, it has distinct regions that are given individual names. For example, the structures that attach the uterus to the pelvic walls (i.e., the broad, cardinal, and uterosacral ligaments) are called the parametria. Similar tissues attach the vagina to the pelvic walls, and these are referred to as the paracolpium (Fig. 1). They are continuous with the cardinal and uterosacral ligaments when the uterus is in situ.

The structures that support the vagina can be divided into three levels that correspond to differing areas of support (Fig. 2 and Table III). The portion of the vagina adjacent to the cervix is suspended from above (level I: suspension) by the relatively long connective tissue fibers of the upper paracolpium. In the midportion of the vagina the paracolpium becomes shorter and attaches the vaginal wall laterally to the pelvic walls (level II: attachment). Near the introitus the vagina is fused laterally to the levator ani muscles and posteriorly to the perineal body while anteriorly it blends with the urethra (level III: fusion) (Fig. 3). In level III there is no intervening paracolpium between the vagina and its adjacent structures, contrary to the situation in levels I and II.

Anatomic observations. The cephalic 2 to 3 cm portion of the vagina constitutes level I. The fibers of the paracolpium suspend this part of the vagina and arise over a broad area originating from the region of the greater sciatic foramen over the piriformis muscles, from the pelvic bones in the region of the sacroiliac articulation, and from the lateral sacrum. These fibers are primarily vertical in orientation (Fig. 4), but some also proceed dorsally from the vagina toward the sacrum in a more horizontal direction when viewed in the standing position (Fig. 5). At the lateral margin of the vagina the fibers of the paracolpium diverge to envelop its anterior and posterior surfaces (Fig. 5). As is true of other visceral ligaments, these suspensory fibers are composed of perivascular connective tissue, blood and lymphatic vessels, and nerves. Within this meshwork there is also a considerable amount of nonvascular smooth muscle.

Structurally, the paracolpium's suspensory fibers in level I converge from their broad origin on the lateral pelvic walls and sacrum to their localized attachment to the lateral walls of the vagina. In effect the upper portion of the vagina hangs from the pelvic walls and sacrum. When downward force is applied to the vaginal apex in an attempt to cause its eversion, the suspensory fibers of the paracolpium prevent this from happening (Fig. 6, *A*). Transecting the paracolpium in this region allows the upper vagina to evert into the lower vagina when downward force is applied (Fig. 6, *B*). Upward traction on the vaginal apex in level I elongates this

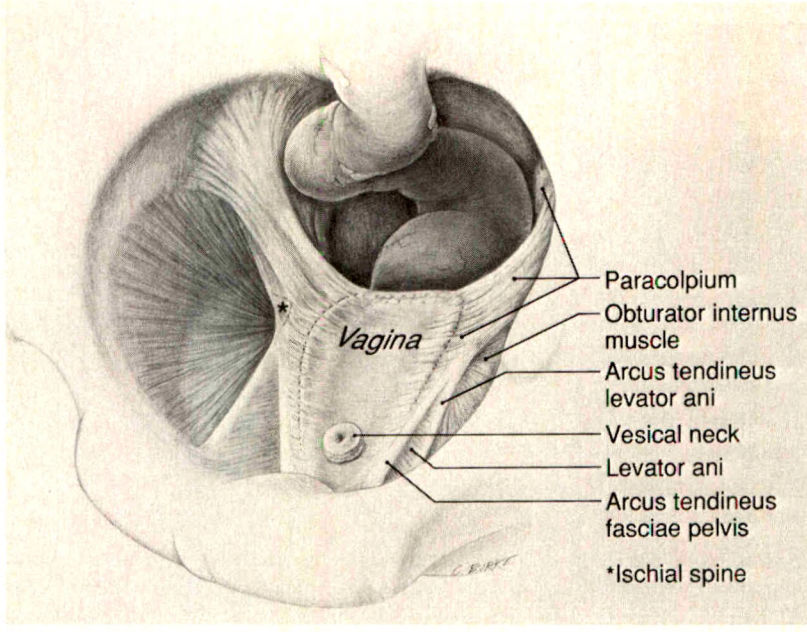


Fig. 1. Vagina and supportive structures drawn from dissection of 56-year-old cadaver after hysterectomy. Bladder has been removed above vesical neck. Paracolpium extends along lateral wall of vagina.

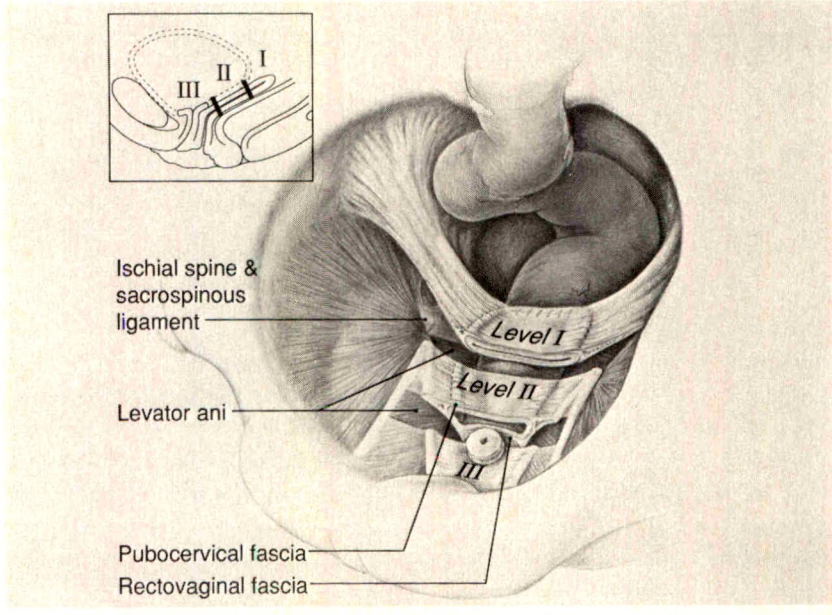


Fig. 2. Level I (suspension) and level II (attachment). In level I paracolpium suspends vagina from lateral pelvic walls. Fibers of level I extend both vertically and also posteriorly toward sacrum. In level II vagina is attached to arcus tendineus fasciae of pelvis and superior fascia of levator ani muscles.

portion of the vagina in a cephalic direction. This motion is not resisted by the suspensory fibers but is limited only by the vaginal wall's ability to stretch.

In level II the vagina comes closer to the pelvic wall, the length of the paracolpium becomes shorter, and the nature of the vagina's connections changes from one of suspension to one of lateral attachment (Fig. 3). The connective tissue that attaches the vagina to the pelvic walls is composed of smooth muscle and a mixture of collagen and elastin. It is more dense than that of the cardinal and uterosacral ligament complex. The blood vessels, lymphatics, and nerves that characterize the

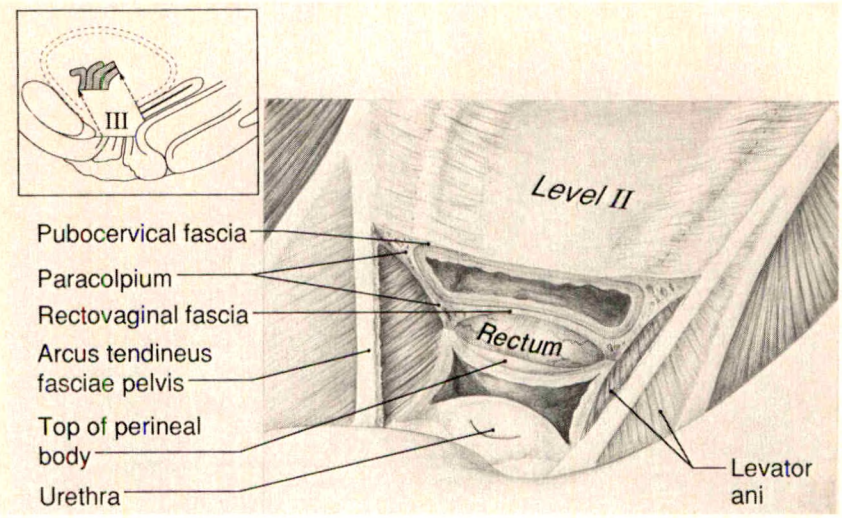


Fig. 3. Detail of levels II and III are shown after wedge of upper urethra and anterior vaginal wall has been removed *(shaded area of inset)*, exposing anterior surface of rectum. In level III vagina is fused to medial surface of levator ani muscles, urethra, and perineal body. Anterior surface of vagina and its attachment to arcus tendineus fasciae of pelvis forms pubocervical fascia, while posterior surface, through its attachment to superior fascia of levator ani muscles, forms rectovaginal fascia.

Table III. Clinical-anatomic correlation

Level	*Structure*	*Function*	*Effect of damage*
Level I: Suspension	Upper paracolpium	Suspends apex to pelvic walls	Prolapse of vaginal apex
Level II: Attachment	Lower paracolpium		
	Pubocervical fascia	Supports bladder and vesical neck	Cystocele-urethrocele
	Rectovaginal fascia	Prevents anterior expansion of rectum	Rectocele
Level III: Fusion	Fusion to perineal membrane, perineal body, musculi levator ani	Fixes vagina to adjacent structures	Urethrocele or deficient perineal body

upper paracolpium continue along the lateral margin of the vagina in this region.

The attachment of each lateral margin of the vagina to the pelvic wall in level II becomes more direct and the paracolpium becomes shorter (Figs. 2 and 5). The connection of the anterior vaginal wall to the arcus tendineus fasciae of the pelvis forms a supportive layer that lies under the bladder and corresponds to the pubocervical fascia (Fig. 3). The posterior wall is attached to the superior fascia of the levator ani muscles and forms the rectovaginal fascia. Although the pubocervical fascia and rectovaginal fascia function as different layers, their continuity along the lateral margin of the vagina can be seen clearly (Fig. 5). In the lower regions of level II (in contradistinction to level I) the connective tissue of the paracolpium extends over both the anterior and posterior surfaces of the vagina in the midline.

The lateral attachments of the vagina to the arcus tendineus fasciae pelvis and superior fascia of the levator ani muscles in level II fix it firmly in place. Downward pressure on the anterior surface of the vagina toward the introitus is resisted by the anterior vaginal wall and its attachments to the arcus tendineus fasciae pelvis (pubocervical fascia). Upward pressure on the posterior surface of the vagina directed toward the introitus is resisted by the posterior vaginal wall and its attachments to the levator fascia (rectovaginal fascia). Unlike the vagina in level I, the vagina in this region cannot easily be moved in a cephalic direction.

Level III corresponds to the region of the vagina that extends from the introitus 2 to 3 cm above the hymenal ring and is fused with the structures that surround it. Unlike in the upper levels, there is no intervening connective tissue of the paracolpium that separates the vaginal wall from adjacent structures (Fig. 7). Anteriorly, the vagina fuses with the urethra and is embedded in the connective tissue of the perineal membrane (urogenital diaphragm). Laterally, it blends with the medial margins of the levator ani muscles. Posteriorly, it fuses with the perineal body. The vagina in this area has no

mobility separate from its adjacent structures. Displacement of the muscles, perineal body, or urethra carries the vagina with it. Lengthening of the levator ani in this area therefore opens the vaginal lumen but does not change the relationship between the vagina and the muscle. Clinically, this region is characterized by the visible contours of adjacent structures. The bulge of the medial border of the levator ani muscles, as well as that of the urethra, can be easily seen as the vaginal wall intimately covers their inner surfaces.

The three levels of vaginal support are continuous with one another and therefore are interdependent. For example, the upper border of level II depends for support on its connection to level I. In this way these portions are part of a continuum, although for the sake of convenience they have been described separately in this report.

Comment

In this study we found that it was not possible to evert the vaginal apex as long as the suspensory fibers in level I were intact. Therefore it would appear that the critical anatomic change in women with vaginal eversion after hysterectomy is failure of these suspensory fibers to hold the upper vagina in its normal position. In a woman who has previously had a hysterectomy, cystocele, or rectocele can develop as a result of problems in level II, but as long as the suspensory fibers of level I are intact, vaginal eversion does not occur. These fibers attach to the region of the vagina adjacent to the scar from the previous hysterectomy, so careful examination of patients with prolapse after hysterectomy should be directed toward determining whether the vaginal apex descends excessively with valsalva pressure or coughing to the level of the hymenal ring. As long as suspension of the vagina in level I is intact, failure of level II supports presents as cystocele or rectocele or both without eversion of the vagina. These cases can be managed with repair of the cystocele and rectocele. However, if the suspensory fibers in level I have failed, vaginal eversion persists immediately after colporrhaphy unless the vagina is resuspended.

The type of prolapse that is present after hysterectomy will depend on which levels of support have failed. Although usually thought of as a generalized process, genital prolapse occurs because of a combination of individual defects that affect localized areas.[11] Defective suspension of the vaginal apex (level I) with preservation of midvaginal support (level II) presents as a simple eversion of the upper vagina. It can be accompanied by downward prolapse of the peritoneum that is attached to the posterior vaginal wall, forming an enterocele, but as long as the level II supports are intact, there is no cystocele or rectocele. In these cases holding the vaginal apex at the level of the ischial spine while the woman strains relieves all visible prolapse, and suspension of the apex without other repair will be curative. This represented 33% of patients with vaginal eversion in our experience.[1]

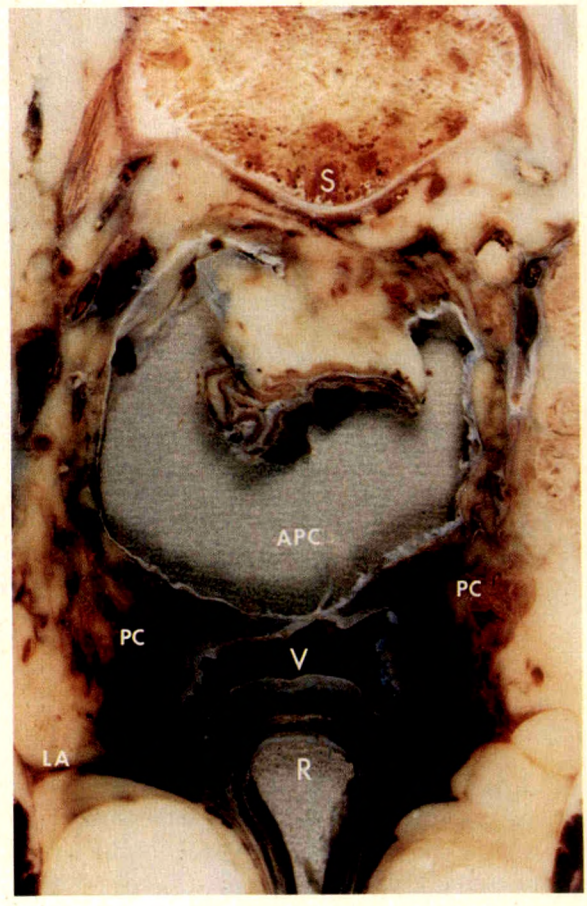


Fig. 4. Coronal section through upper vagina (level I) in 26-year-old cadaver that had previous hysterectomy, showing vertical orientation of paracolpium *(PC)* as it suspends vagina *(V)* above rectum *(R)* and levator ani *(LA)* muscles and below abdominopelvic cavity *(APC)*. *S*, Sacrum.

When the vaginal apex is no longer suspended and failure of the level II supports (pubocervical or rectovaginal fasciae) is also present, then vaginal eversion is associated with either a cystocele or a rectocele or both. This is referred to as complex vaginal eversion, whereby prolapse of the apex is accompanied by a cystocele or rectocele, and represents 67% of vaginal eversions.[1] Among these patients 7% have apical prolapse with cystocele and 30% have apical prolapse with rectocele, whereas 30% have both cystocele and rectocele along with vaginal eversion. In these individuals cystocele or rectocele or both are still present when the apex is held at its normal position during examination.

The attachments of the distal vagina to the medial margins of the levator ani muscles in level III are so dense that the lower portion of the vaginal wall always

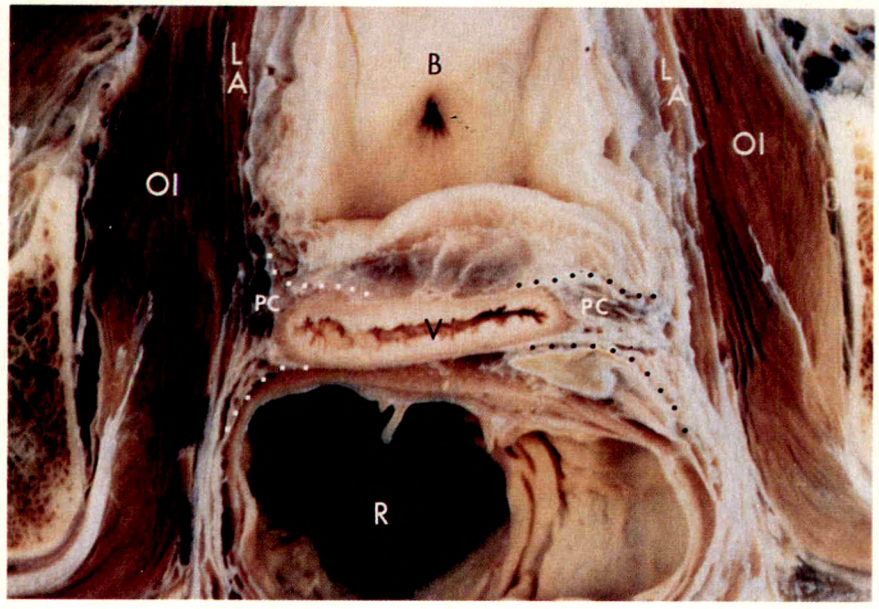


Fig. 5. Transverse whole-pelvis section of 14-year-old nulliparous cadaver. Asymmetry of specimen shows level I with long segment of paracolpium *(PC)* between vagina *(V)* and lateral pelvic wall on right *(black dot outline)*. Direct attachment in level II on left *(white dot outline)*. *B*, Bladder; *LA*, levator ani muscles; *OI*, obturator internus; *R*, rectum.

remains attached in this region. The importance of this region has to do more with the ability of the levator ani muscles to keep the vagina closed than with the vagina's ability to remain attached to its surrounding structures. Certainly, damage to the perineal body and urethral supports in this region is important, but as yet this is poorly understood.

Considering the importance of the suspensory fibers in level I, how can this information be used to help prevent vaginal eversion after hysterectomy? In the normal individual the uterus and upper vagina are suspended by the continuous support offered by the parametrium (cardinal and uterosacral ligament complex) and the paracolpium in level I. This sheet of tissue is approximately 6 cm wide. During abdominal hysterectomy the cardinal ligaments and uterosacral ligaments are separated from the cervix, leaving only 2 to 3 cm of the paracolpium attached to the vagina. Furthermore, if transection of the paracolpium from the vagina is continued past the cervix for another 1 to 2 cm, then little of the level I fibers remains to support the vagina. This diminution of the suspensory fibers may well predispose patients to vaginal eversion later in life as the general deterioration in connective tissue occurs with age.

Anatomically, this area can be strengthened during hysterectomy not only by reattaching the cardinal and uterosacral ligaments to the vaginal apex but also by performing intrafascial hysterectomy techniques[12] that avoid detachment of any of the paracolpium from the vagina. This would allow the entire 6 cm of cardinal ligament and paracolpium to participate in support of the vaginal apex. The fact that vaginal eversion does not immediately follow detachment of the paracolpium from the vagina attests to the redundancy of support in normal women. However, women who have undergone hysterectomy and in whom parts of the suspensory apparatus were not reattached may be at increased risk for subsequent vaginal eversion because age weakens their supportive tissue. This could explain the fact that vaginal eversion develops in equal numbers of patients after abdominal and vaginal hysterectomy,[4] even though patients who undergo vaginal hysterectomy are more likely to have previously had prolapse. Improvement of vaginal suspension is consistent with traditional techniques that achieve elevation, in addition to obliterating the cul-de-sac,[13] and help to reestablish the normal depth and axis of the vagina.[14]

It is interesting that the difference in attachment of the vagina to the pelvic walls between levels I and II and level III parallels the different embryologic derivations of these regions. Levels I and II are derived from the müllerian ducts and have attachments to the lateral pelvic wall similar to those of the uterus and tubes. The lower portion of the vagina, the portion that is present in patients with müllerian agenesis (level III), comes from the urogenital sinus and does not have the paracolpium between it and adjacent structures.

It should be emphasized that this article discusses only one part of the multifaceted structure of the pelvic

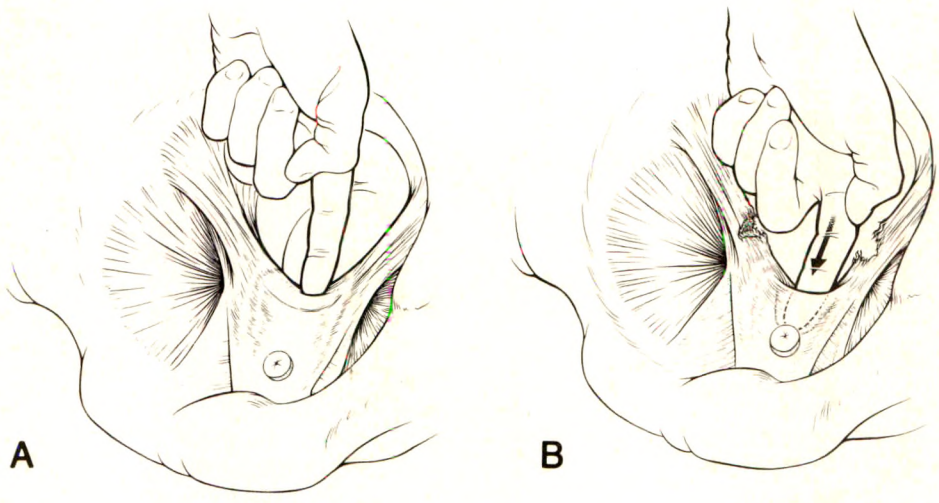


Fig. 6. A, When force is applied to vaginal apex, eversion of vagina is resisted by suspensory fibers of level I. **B,** Transecting fibers of level I permits eversion of vagina to occur.

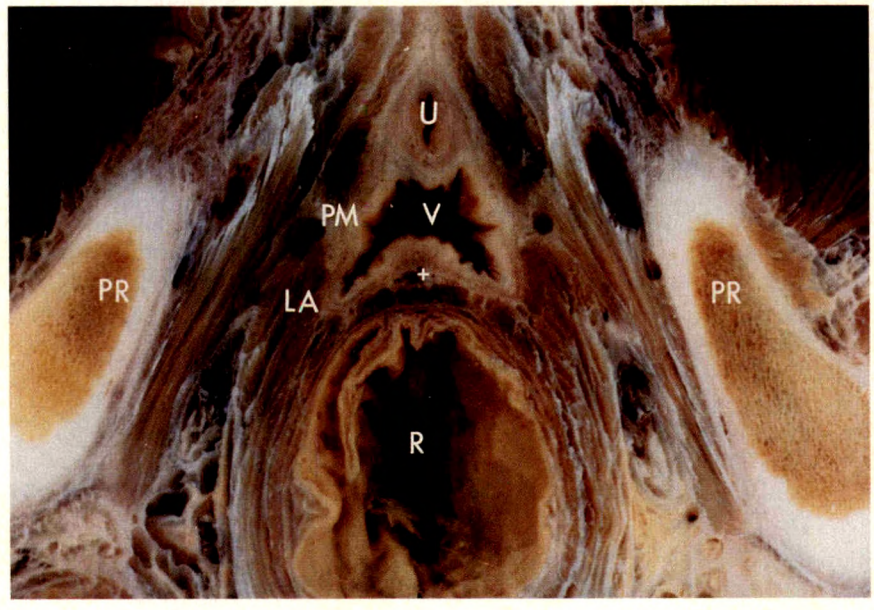


Fig. 7. Transverse section from cadaver shown in Fig. 5 at level III showing fusion of the vagina *(V)* to urethra *(U)*, perineal membrane *(PM)*, levator ani muscles *(LA)*, and top of perineal body (+). *PR*, Inferior pubic ramus; *R*, rectum.

floor. Many factors in addition to the connections of the vagina to the pelvic walls are crucial to pelvic support. The treatment of this one aspect of support in isolation does not imply that it is the only important element involved. Other factors such as damage to the levator ani muscles with resultant increased inclination of the levator plate[15] and alteration of the axis of the vagina[14] are also involved. Further objective studies comparing the anatomy of patients with prolapse with that of those with normal support will be necessary to clarify the relative importance of each of these factors in the development of prolapse. The current report, along with other anatomic investigations into the functional anatomy of the pelvic floor, should prove helpful in directing such studies.

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Discussion

Dr. Stephen H. Cruikshank, Minneapolis, Minnesota. Dr. DeLancey has presented an excellent anatomic description of the three known levels of vaginal support. He has pioneered a single study that microscopically delineates these different levels of support and the sequelae to each level, should a defect occur. The Abstract, Introduction, Material and Methods, and Results sections of his manuscript clearly fit the style of a well-written scientific article; however, I would like the audience and readership to concentrate on the Comment section and Dr. DeLancey's conclusions.

In his study Dr. DeLancey microscopically documented what we have studied, discussed, and clinically concluded no less than four times over the past 5 years at the Central Association meetings. He proved it anatomically using cadavers. He states that his work supports the claims others have made clinically. I would offer here a word of caution. I offer my support to this study, which is excellent in experimental design and concept. I believe it has gotten to the basis of our clinical practice, which is well documented in the literature.[1-4] However, we should never rely entirely on the results of one study; it is hoped that there will be more studies, reproducing these results of Dr. DeLancey and others.

He has examined a group of cadavers, which do not have the same variables as living patients undergoing procedures (we must take into consideration the style of a surgeon, operator knowledge of anatomy, tissue structure of each patient, underlying diseases, etc.).

Dr. DeLancey has eliminated these variables. The controversy that Dr. DeLancey alludes to in the Comment section is indeed created by these factors. Dr. DeLancey claims that the immersion method eliminates distortion, but he has not compared results with this method to those of a living clinical control group.

Specifically, I would like to address three conclusions that stood out in his discussion. He stated: (1) ". . . this area can be strengthened during hysterectomy not only by reattaching the cardinal and uterosacral ligaments to the vaginal apex but also by performing intrafascial techniques. . . ." (2) ". . . women . . . in whom parts of the suspensory apparatus were not reattached may be at risk for subsequent vaginal eversion." (3) "Improvement of vaginal suspension is consistent with traditional techniques that achieve elevation, in addition to obliterating the cul-de-sac, and help to reestablish the normal depth and axis of the vagina." Although Dr. DeLancey concludes these points clearly, they are not new to our profession. However, I am particularly pleased to draw attention to these statements for these conclusions are the same ones that followed studies also presented here in the past.[2-4]

I have three questions that I would like Dr. DeLancey to answer: (1) Clinically, in the office and/or operating room, do you examine patients with the uterus in situ to decide which patients are at possible risk for future vaginal eversion, and how? (2) If you find evidence clinically that a patient has partial defects of the vaginal apex with the uterus in situ, to what extent will you go in your repair to prevent posthysterectomy prolapse? (3) In those patients found to have moderate to severe vaginal eversion with an in situ uterus, what prophylactic methods (prophylactic means to prevent, to ward off) would you use to fully correct the defect of level I suspension?

Dr. DeLancey, I congratulate you. You are a pioneer, truly focused. Aside from the above-mentioned points, you have pioneered a study that has proved microscopically the clinical anatomy of an infrequent, yet tragic (for the patient) complication of hysterectomy—vaginal vault eversion.

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Dr. G. Rodney Meeks, Jackson, Mississippi. I congratulate Dr. DeLancey on his excellent article. He has devoted a significant portion of his career to helping us understand the anatomy and function of pelvic floor support. Dr. DeLancey has a unique opportunity to evaluate histologic and whole-pelvic cross sections. Because of constraints imposed by limited availability of

cadavers, most of us cannot reproduce such basic studies. This article makes a very complex subject understandable. This is accomplished by clear thinking about the subject, concisely written descriptions of the anatomy, thoughtful conclusions, and the use of excellent figures.

The study design is one of simple observation, a technique that is common when surgical and/or anatomic questions are evaluated. No attempt has been made to quantify the degree of vaginal eversion in the specimens nor has the force required to produce prolapse of the vagina been discussed. The question, "How many fibers of the paracolpium can be damaged or lost before prolapse occurs?" has not been addressed. The specimens were studied in an upright position, which may remove some of the potential artifacts.

Describing three levels of vaginal support gives to clinicians a mechanism to explain pelvic floor defects. Furthermore, the information may provide a rationale for selecting a surgical repair technique. It may help to identify patients in whom vaginal vault suspension is appropriate. Could it vindicate Dr. Cruikshank's stance[1] of "prophylactic suspension"? Whereas this presentation is accurate on the basis of my clinical experience, could the conclusions be biased? That is, could the anatomic descriptions in this article reflect what is used clinically and thus justify a specific surgical approach without proving that these anatomic structures are responsible for support? Dr. DeLancey has addressed the question that he proposed in regard to what differentiates women with vaginal prolapse from women with cystocele, rectocele, and enterocele alone. The answer is loss or damage of supporting structures. I remain confused in regard to ways to lessen the likelihood of prolapse after hysterectomy. He has not provided a "how to" for surgeons. Several specific questions may clarify these points: (1) Can this description of vaginal support help clinicians to recognize patients at risk for prolapse? (2) Do partial defects occur? If so, to what extent should one go to identify these defects? (3) What measures can be taken to prevent pelvic floor defects after hysterectomy? (4) Does leaving the vaginal cuff open at the time of hysterectomy have any effect on the risk of vaginal prolapse? (5) Does a closure technique that reapproximates the cardinal and uterosacral complex to the midline have any effect on the risk of vaginal prolapse? (6) If the fascia surrounding the vagina and the cardinal-uterosacral complex are important to support the cuff, would a subtotal hysterectomy, leaving the cervix and its attachments intact, minimize surgical damage and thus reduce the likelihood of prolapse? (7) Does cul-de-sac obliteration have any role? (8) Does the hiatus levator ani have any impact at all on level I and II defects?

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Dr. Christopher J. Jolles, Salt Lake City, Utah. I applaud Dr. DeLancey and his efforts to understand the anatomic principles relating to the operations that we perform, for indeed we are surgeons and are responsible for understanding anatomy and the physical basis of what we do.

During a radical hysterectomy, we dissect the uterosacral ligaments from their origin in the posterior aspect of the cervix down to the window joining the pararectal and rectovaginal space. The entire ligament is clamped and divided, and no effort is made to reattach remnants of this ligament to the vaginal vault.

Frankly, I have never seen a significant vault prolapse in a patient who has undergone a radical hysterectomy. Therefore I wonder what impact this factor has on our understanding of the necessity of paracolpial support to prevent vault prolapse.

Dr. Lane J. Mercer, Chicago, Illinois. Dr. DeLancey, I also want to congratulate you for your fine work and article. I want to point out, perhaps because I am a bit of a hometowner, that Dr. Curtis at Northwestern University described something called a pararectal fascia, which also describes the tissue you are calling the paracolpium. He later developed a suspension of the vaginal apex after vaginal hysterectomy by use of this tissue, which has proved successful.

Long-term experiences have shown that this suspension at the conclusion of vaginal hysterectomy provides results at least as good as those we now attribute to the sacrospinous ligament suspension. I would appreciate your comments about that.

Second, using your description of various levels of vaginal defects, we are seeing posthysterectomy vaginal eversion treated all too often with a level I correction, that is, a sacrospinous ligament suspension, and the level II defect addressed with an anteroposterior colporrhaphy, which does little except to decrease or tighten the caliber of the vagina. It does nothing to suspend it. I would appreciate your comments about how you would handle such patients on the basis of your anatomic findings.

Dr. Kenneth Webster, Cleveland, Ohio. I would like to reiterate Dr. Jolles' comments in that, as gynecologic oncologists, we have a living role model for each case of pelvic dissection. Once again, if the entire uterosacral ligament is removed along with the uterus and upper vagina, as well as the cardinal ligament, there is nothing to resew to the apex of the vagina. In 21 years of postoperative experience, I have never seen a vault prolapse in a patient undergoing radical hysterectomy.

Dr. Suzanne R. Trupin, Champaign, Illinois. I was just curious about whether this patient population was premenopausal or postmenopausal and if the author could comment on whether the pelvic support ligaments are significantly changed in these populations.

Dr. Maurice J. Webb, Rochester, Minnesota. Congratulations on an elegant study. The aspect I would question is that you are concentrating on the lateral supports of the vagina. You did mention that the vagina

incorporated pubocervical and rectovaginal fascia. Would you comment on the thought that possibly some of the weakness that develops in prolapse is not in the lateral supports but is due to the central weakness of this fascia, which allows the protrusion to occur?

PRESIDENT YOUNGBLOOD, Kansas City, Missouri. I had the privilege of reviewing this article. You made a clear statement that intrafascial hysterectomy would seem to be the way to go as far as technique is concerned. You didn't mention that today, and I wonder if you would comment on that.

DR. DELANCEY (Closing). I thank both Dr. Cruikshank and Dr. Meeks for their thoughtful and probing discussion. They have raised several very important issues. I will go through them in the order in which they were presented.

I would agree with Dr. Cruikshank's comment that it is very difficult to know about the distortions that occur in an anatomic specimen when compared with a living individual. The most profound one comes from the lack of contractility of the levator ani muscle, and that is a limitation in all anatomic studies and at the present time cannot be overcome. However, if you keep that in mind while dissecting and don't overinterpret what you see, then it is possible to look at the connective tissue (which tends to change relatively little after death) with some accuracy. The potential distortions are important, and we must keep them in mind when interpreting the results of these anatomic studies.

We actually did compare the spatial relationships in cadavers fixed by immersion with measurements that are available from living individuals. As mentioned in the manuscript, there is a book by Dickinson that was published in the 1950s that contains measurements of pelvic anatomy in living women, and we were able to document the fact that the immersion technique resulted in the same topography that was found in the living individual. Certainly, in the unfixed cadavers there are no similar data with which to compare.

The next issue that Dr. Cruikshank raised, and I think it is a critical one for us to consider, is the fact that there is a large difference between what I present as anatomic findings and what is presented in a discussion of clinical applications of these findings, the latter being a largely philosophic discussion.

I have no specific data to present concerning the suspension of the vagina at the time of abdominal or vaginal hysterectomy. The discussion of this issue deserves the kind of careful study that Dr. Cruikshank has done in his articles in which he carried out follow-up of patients after various suspension techniques. The wisest course for us to follow is to look at the anatomy of vaginal support for an approach to supporting the vaginal apex but to actually depend on clinical follow-up studies of different techniques to gain data on the success of those different suspension techniques.

Dr. Cruikshank also asked about the office evaluation of a patient to detect the degree of apical prolapse that is present with or without the uterus in situ. We do this in both the supine and the standing position. The hymenal ring is a reliable reference point, and you can simply measure the distance of the scar at the vaginal apex relative to the hymenal ring, either above it or below it, to document the degree of apical prolapse that is present once the prolapse has maximally developed.

He also asked about partial defects in the support of the vaginal apex, which is a very important issue. The status of vaginal support is not a yes-or-no question, it's a "how much" question. It is not, is the vagina prolapsed? It is, how far down is the apex relative to the position that it should normally be in (which is approximately 4 to 6 cm above the hymenal ring in the resting individual).

Our quantitative studies on cervical descent[1] revealed that the lateral margin of the cervix can be pulled to the level of the hymenal ring in the normal, anesthetized, multiparous woman. In interpreting the normal support of the vaginal apex under traction, we need to gather some data about this by taking asymptomatic patients who have previously undergone hysterectomy and ascertaining the location of the vaginal apex. I think that Dr. Cruikshank is right, that there are partial defects that occur. Sometimes the vaginal apex is well supported; other times it's obviously everted, and then there's a large group of patients who are in the middle with varying degrees of descent.

Dr. Cruikshank also raises the issue of patients who have moderate to severe uterovaginal prolapse. In these individuals tenaculum traction may cause the cervix to protrude, for example, 4 cm below the hymenal ring. The vagina is attached to the cervix, and he points out that the vagina prolapses as well.

There are a number of other techniques that have been proposed to resuspend the vagina in these patients, which are performed in conjunction with vaginal hysterectomy. At present one can only speculate on the importance of any one of these techniques because there aren't sufficient data to be able to discriminate between the long-term outcomes of various resuspension techniques.

Dr. Meeks has asked if we can identify the patients who are at risk for prolapse. This could mean one of two groups: (1) the patients who have true prolapse that is undetected or (2) those in whom prolapse may develop in the future but who are normal now. I would comment that at present, because prolapse is a non-life-threatening condition and many women have asymptomatic relaxation, we should focus not on prognosis except for the purposes of scientific studies but rather on the important diagnostic aspect of detecting prolapse when it is present. It is important to know where the apex of the vagina is in the woman who has defective support after hysterectomy. If an inadequate preoperative examination is performed and the patient isn't straining forcefully enough, a vaginal eversion that is present will be undetected. Anterior and posterior colporrhaphy performed under the presumption that this is a simple cystocele and rectocele will fail to correct

the prolapse unless something is done to resuspend the vagina at that time. I would emphasize this as a diagnostic and not prognostic issue.

Dr. Meeks also raised the question of partial defects that we've already addressed and then again asked about the management of the vaginal cuff at the time of hysterectomy, specifically raising the issue of whether leaving the cuff open adds to support of the vagina, assuming that it will become adherent to the structures that surround it. This mechanism for support has been discussed for many years. It seems to me anatomically implausible because the ligaments retract fairly quickly after you release them and, unless they are reattached to the cuff, cannot be expected to seal to it.

Tying the ligaments together in the midline to resuspend the vaginal apex is a technique that comes from the concept that the suspensory ligaments are transverse, as implied by the term *transverse cervical ligaments*. The concept that the ligaments run horizontally from the pelvic sidewall inwardly toward the cervix is a misapprehension that comes from looking at cross-sectional material. When we made three-dimensional reconstructions of the anatomy in this area, it was obvious that the fibers of the cardinal ligaments are vertical in the standing individual. So it is illogical to pull things together in the midline because normally those ligaments do not come to the midline but attach vertically to the lateral margins of the vagina and the cervix.

A supracervical hysterectomy, by preserving the attachments of the ligaments to the genital tract, might help to maintain support of the genital tract, but I think the significant and continuing problems with cervical carcinoma would outweigh the considerations about genital prolapse, especially since we have relatively good treatment for genital prolapse and less satisfactory treatment for malignant disease of the cervix.

A cul-de-sac obliteration remains an important part of our surgical management in these individuals, although we have few specific data concerning that. Our approach is to focus on the fibrous attachments, and we have not considered the cul-de-sac separately.

The hiatus of the levator ani muscle is of importance, and the function of the levator ani muscle itself is also critical. I believe that the levator ani muscles are more important than the fibrous connections of the vagina to the pelvic sidewall. We are just beginning to gather data on this condition. It is something that Halban and Tandler[4], who did the most extensive studies of the pathophysiologic characteristics of genital prolapse, maintain very forcefully throughout their work, and I am starting to believe that, in fact, the levator ani muscle is the primary structure responsible for normal support. Today I have focused on the fibrous supports to define them, but I believe that over the course of the next several years we will begin to better appreciate the importance of the levator muscles to support.

Dr. Jolles raised the issue of the detachment of paracolpium from the vagina during radical hysterectomy and asks why this doesn't lead to prolapse. It is also true that at the same time an upper vaginectomy is performed. The portion of the vagina that depends on level I suspension is removed along with the radical hysterectomy specimen as an upper vaginectomy; that portion of the vagina that would be unsupported after detachment of these ligaments is therefore no longer there to prolapse. This explains the fact that there is no vaginal prolapse afterward.

The other issue in these patients concerns the fact that the levator ani muscle probably functions normally in patients with cervical cancer, and so there's much less downward force on the fibrous connective tissue because the pelvic floor remains closed through normal muscular activity. Further studies of individuals in whom the detachment occurs but the upper vagina is not excised might be a very good way for us to test some of these issues.

Dr. Mercer brought up the concept of the pararectal fascia as a supportive element. When you become interested in the endopelvic fascia as I have, it is inevitable that you collect a long list of terms for the endopelvic fascia. There are probably 70 different names that have been applied to this one body of connective tissue, and pararectal fascia is one of the terms that occurs commonly.

The endopelvic fascia is a continuous tissue that has various regions. When someone studies it and becomes interested in a particular region, they are naturally drawn toward the idea of inventing their own name for it. The paracolpium actually is an established anatomic term that has been more popular in the German anatomic studies. The pararectal fascia is the posterior extension of the connections of the vagina, and there are a number of reports using that fascia which overlies the iliococcygeus muscle for resuspension. That technique does seem to be effective.

The second question that Dr. Mercer raised has to do with separate consideration of level I and level II at the time of treatment of vaginal eversion. I would agree with Dr. Mercer that the problems of resuspending the vagina (level I) are ones that are successfully accomplished with several techniques, including sacrocolpopexy, sacrospinous ligament suspension, and some other modifications of those.

The current problem is not with achieving good apical support. It's the cystocele (level II) that develops afterward, as Shull et al.[2] described. The concept that a cystocele develops because the pubocervical fascia stretched is an entirely unproved concept. Goff,[3] in the 1940s, examined the then-current hypothesis that it was the thinning of the pubocervical fascia that was responsible for cystourethrocele. He found, when he looked at the pubocervical fascia in individuals with cystocele-urethrocele, that it was actually thicker than in the normal individual not thinner as most people suppose.

This very important question raises the issue of the scientific study of pelvic floor function and pelvic floor support. With the armamentarium of imaging tech-

niques and the sophisticated physiologic measuring instruments available we can begin to get out of the arena of opinion about these issues and start to collect data that can answer some of these important clinical questions that have been raised today.

I think that it was Dr. Trupin who mentioned the important question of premenopausal and postmenopausal changes. I didn't have time to detail the ages of our cadavers, although they are included in the original manuscript. The age range was from 7 months to 104 years. The mean age of the cadavers was the mid-50s.

It is certainly possible to see changes that occur in the connective tissue with age. It becomes much less robust and much more fragile. There didn't seem to be precipitate change with the menopause, but there did seem to be quite a dramatic change over the course of examining both young and old cadavers. That's another area that we will be able to collect data on over the course of the next 5 or 10 years.

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Receiver-operator characteristic, efficiency analysis, and predictive value of serum progesterone concentration as a test for abnormal gestations

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OBJECTIVE: Our objective was to determine if a disciminatory progesterone concentration could be established that confidently predicted abnormal early gestations.

STUDY DESIGN: We analyzed differences in progesterone concentrations between normal (n = 40) and abnormal (n = 34) pregnancies during the first 49 days of gestation. The receiver-operator characteristic curve, test efficiency, and predictive value of serum progesterone to discriminate between an abnormal and normal first-trimester gestation were calculated for progesterone concentrations between 5 and 25 ng/ml.

RESULTS: Receiver-operator characteristic curve analysis indicated that the best discriminatory progesterone concentration was 10 ng/ml. Test efficiency was maximum between serum progesterone concentration of 9 to 14 ng/ml (80%). When progesterone was <10 ng/ml, the predictive value of the abnormal test result was >90%.

CONCLUSION: Receiver-operator characteristic analysis, test efficiency, and the predictive value of an abnormal test result suggest that the best progesterone cut off point that predicts abnormal early pregnancies is 10 ng/ml. (Am J Obstet Gynecol 1992;166:1729-37.)

Key words: Receiver-operator characteristic, efficiency analysis, progesterone, abnormal gestation

Low serum progesterone concentrations are associated with abnormal (abortive and ectopic) gestations. However, the specific concentration of serum progesterone that represents the best discriminator of an abnormal gestation is unknown. Johansson[1] was the first to demonstrate that abnormal early gestations had progesterone concentrations lower than those of viable intrauterine pregnancies. More recent studies have expanded and quantitated these observations. Matthews et al.[2] and Yeko et al.[3] reported that serum progesterone concentrations of ectopic pregnancies were <15 ng/ml and did not overlap with the distribution of concentrations of normal intrauterine pregnancies. Furthermore, few intrauterine abortions had progesterone concentrations ≥15 ng/ml. Hubinot et al.[4] reported less uniform results, noting that 11% (64/578) of normal gestations had a concentration <15 ng/ml. Stovall et al.[5] screened 582 emergency room admissions for early pregnancy complications with a single serum progesterone measurement and observed that 13 of 67 ectopic pregnancies (19%) had a concentration ≥15 ng/ml, whereas 11% of the normal pregnancy population had a concentration <15 ng/ml. Thus the optimal discriminatory progesterone concentration that can diagnose abnormal pregnancies is not well established. As a result, its potential accuracy in predicting abnormal first-trimester gestations is unclear.

For this reason we tested the efficacy of a wide range of progesterone values in discriminating between normal and abnormal pregnancies during the first 49 days of gestation. These data were analyzed by statistical methods that evaluate the performance of the test (predictive value and test efficiency) and the detection rates of conditions within the study population (sensitivity). In this way, decisions concerning the ability of serum progesterone measurements to detect abnormal pregnancies can be based on the performance of the test rather than an arbitrary assignment of cut-off points.

Material and methods

Patients. From August 1985 to April 1991, 74 asymptomatic pregnant patients seen at University Hospital

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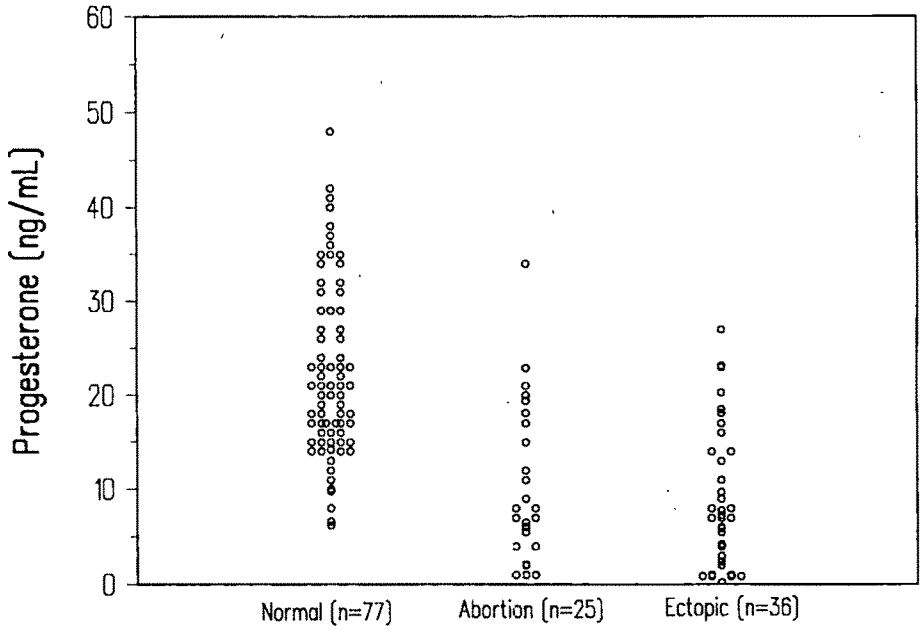


Fig. 1. Distribution of progesterone concentrations in women with normal, abortive, and ectopic pregnancies during first 49 days of pregnancy.

Table I. Summary of number of patients, observations, and mean progesterone concentration by condition of first-trimester gestations

Condition	*No. of patients*	*Observations*	*Progesterone (ng/ml)*	*SEM*
Normal	40	77	24.1	1.5
Aborted	16	25	10.5	1.7
Ectopic	18	36	9.0	1.2

between 28 and 49 days from last menstrual period (LMP) who were judged to be at risk for ectopic pregnancy had serum progesterone measurements one to three times per week until diagnosis. Risk was assigned on the basis of previous history of infertility, tubal or abdominal surgery, or previous ectopic pregnancy. Serum progesterone concentrations were determined by radioimmunoassay with a commercially available kit (Diagnostic Products Corporation, Los Angeles, Calif.). The intraassay and interassay coefficient of variation was 8% and 10%, respectively. All patients conceived spontaneously. Patients were classified into three groups on the basis of the final diagnosis: group 1, normal pregnancy (n = 40); group 2, abortive intrauterine pregnancy (n = 16); and group 3, ectopic (tubal) pregnancy (n = 18). Twenty-one concentrations of progesterone (5 to 25 ng/ml at 1 ng/ml increments) were used to test critical levels that best determined normal and abnormal results.

Statistics. The capacity of a given progesterone level to discriminate between abnormal (ectopic and abortive) and normal pregnancies was determined with 2×2 contingency tables to calculate the predictive value, test efficiency, sensitivity, and specificity at specific progesterone concentrations.[6] The contingency table had the following structure:

	Abnormal condition	*Normal condition*
Abnormal test	a	c
Normal test	b	d

The abnormal test result is the predictive value of the test to determine an abnormal condition when progesterone is below the tested concentration $(a/a + c)$, and the normal test result is the probability of a normal condition when progesterone is above the tested concentration $(d/d + b)$. The normal and abnormal test result were individually correlated with serum progesterone. The test efficiency is the proportion of patients correctly assigned as either normal or abnormal $(a + d/a + b + c + d)$. Test efficiency was also correlated with progesterone concentrations. A receiver-operator characteristic curve was used to determine the best discriminatory progesterone concentration by plot-

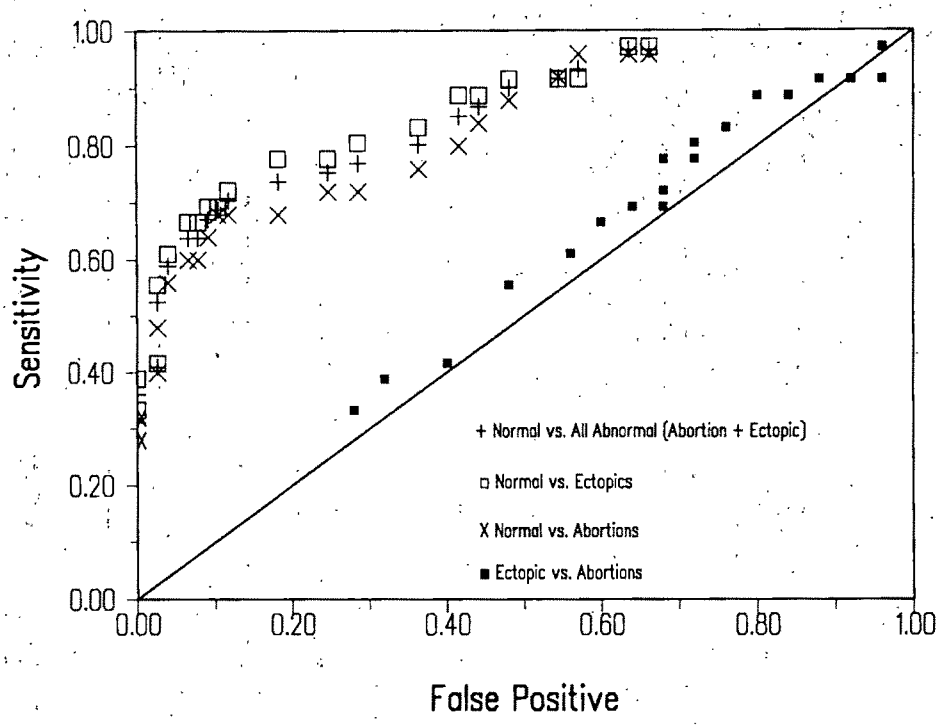


Fig. 2. Receiver-operator characteristic curve. This analysis correlates sensitivity of test with false-positive rate (1 − specificity) for each progesterone concentration tested. Analysis was performed comparing normal pregnancies against all abnormal pregnancies (+), intrauterine abortions (×), and ectopic pregnancies *(open box)*. Additionally, receiver-operator characteristic curve of ectopic pregnancies compared with abortions is plotted *(block)*. *Diagonal line* drawn from 0.00 to 1.00 represents "worst" curve.

ting the sensitivity $(a/a + b)$ of the test at each progesterone level against the percent of normal subjects incorrectly classified as abnormal (false positive = 1 − specificity $(d/d + c)$). The benefit of predictive value and test efficiency calculations are their ability to estimate the performance of the test. The value of the receiver-operator characteristic analysis lies in its ability to provide a clear computational model of the test over the full range of discriminatory progesterone concentrations and estimate the best or discriminatory cutoff for the test. The mean serum progesterone concentrations of each group were compared with a *t* test.

Results

Table I summarizes the number of patients, the number of serum progesterone values, and the mean serum progesterone concentration in each diagnostic group. The average progesterone concentration in women with a normal gestation was 24.1 ± 1.5, intrauterine abortion was 10.5 ± 1.7, and ectopic pregnancy was 9.0 ± 1.2 ng/ml (mean ± SEM). The mean progesterone concentration in normal gestations was significantly higher ($p < 0.001$) than the progesterone of either ectopic or abnormal intrauterine gestations. The mean progesterone concentration in women with abnormal intrauterine gestations was not significantly different ($p = 0.268$) than that in women with a tubal pregnancy. These data are graphically represented in Fig. 1.

Fig. 2 compares the receiver-operator characteristics curves for normal pregnancies against abnormal gestations (abortion and ectopic), abortions only, and abortion versus ectopic pregnancy at 21 progesterone concentrations. This analysis demonstrates a favorable improvement in the true-positive rate (sensitivity) and minimal inclusion of false-positive results when progesterone concentrations are between 5 and 10 ng/ml for normal pregnancy comparisons. When the discriminatory progesterone is >10 ng/ml, the curve becomes flat. This indicates that a discriminatory progesterone >10 ng/ml provides little improvement for the detection of true-positive conditions, but only includes more false-negative results. Additionally, the receiver-operator characteristics curve for abortions and ectopic pregnancies *(solid block)* indicates that these conditions are indistinguishable from one another on the basis of progesterone testing. The slope of the linear regression is 1.05, and the correlation coefficient is 0.986. A receiver-operator characteristics curve that is parallel to

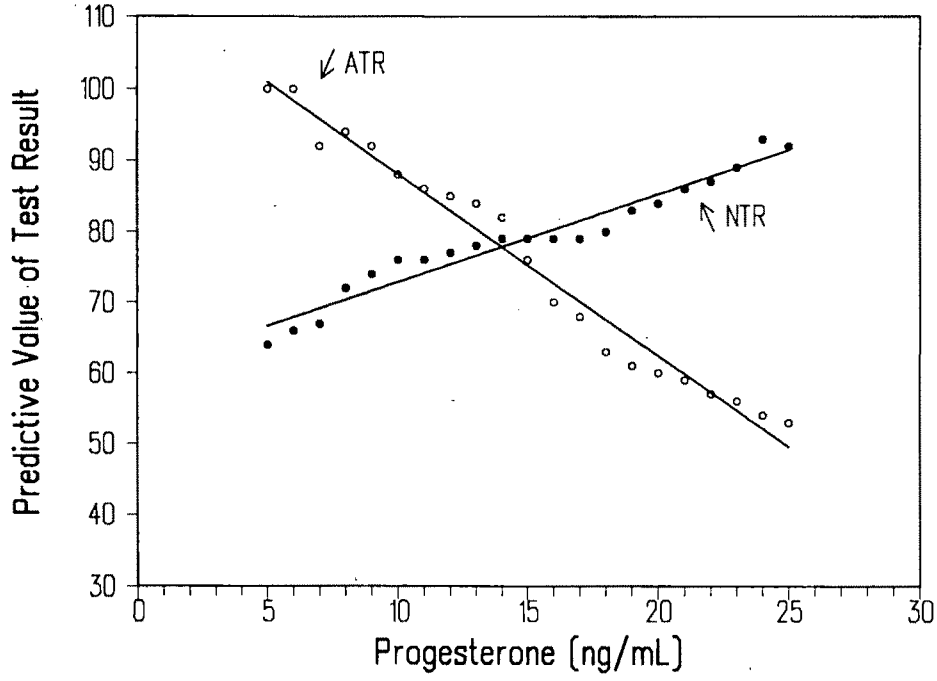


Fig. 3. Predictive value of abnormal test result *(ATR)* and normal test result *(NTR)* compared with discriminatory serum progesterone concentration in women with normal or abnormal gestations.

Table II. Predictive values of test results

Progesterone cutoff value (ng/ml)	*Predictive value of normal test result: Normal vs abnormal (%)*	*Predictive value of abnormal test result: Normal vs abnormal (%)*
5	64	100
6	66	100
7	67	92
8	72	94
9	74	92
10	76	88
11	76	86
12	77	85
13	78	84
14	79	82
15	79	76
16	79	70
17	79	68
18	80	63
19	83	61
20	84	60
21	86	59
22	87	57
23	89	56
24	93	54
25	92	53

The normal test result represents the ability of the test (progesterone concentration) to predict the normal condition when progesterone is above the cutoff level. The abnormal test result represents the ability of the test to predict the abnormal condition when progesterone is below the cutoff level. Calculations were performed at 21 progesterone concentrations (5 to 25 ng/ml) and normal pregnancies were compared with abnormal pregnancies (aborted and ectopic).

a diagonal line drawn from 0,0 to 1,1 represents a poor (worst) test, having no ability to detect either condition.

Because abortions could not be distinguished from ectopic pregnancies, our analysis of predictive value and test efficiency focused on testing for normal pregnancies compared with abnormal conditions (abortion and ectopic). Table II summarizes the normal and abnormal test result for normal gestations compared with abnormal gestations for progesterone concentrations betweeen 5 and 25 ng/ml. The abnormal test result for normal versus abnormal pregnancies ranged from 53% to 100% over the range of serum progesterone con-

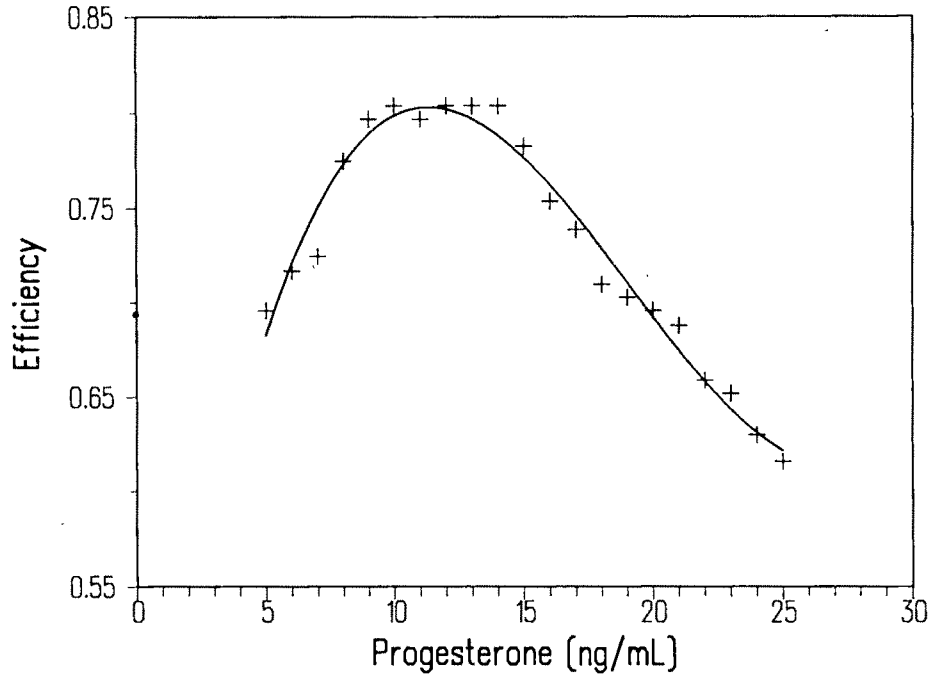


Fig. 4. Correlation of test efficiency (proportion of cases correctly classified either abnormal or normal) with serum progesterone. Second-order polynomial describes these data ($y = 0.353 + 0.093x - 0.006x^2$, $r = 0.986$).

centrations tested. The progesterone concentration tested was negatively correlated with the abnormal test result (Fig. 3). The linear regression of the abnormal test result for abnormal gestations demonstrated a significant negative correlation with increasing progesterone concentrations ($r = -0.988$).

The normal test result of the progesterone discriminatory test is also summarized in Table II. Just like the abnormal test result, these calculations compared normal versus abnormal gestations at 21 progesterone concentrations. The normal test result was a poor predictor of a normal pregnancy and was not ≥80% until the progesterone concentration was ≥18 ng/ml. Fig. 3 shows the correlation between the normal test result derived from these calculations with progesterone concentrations. It was positively correlated with increasing progesterone concentrations ($r = 0.972$).

Fig. 4 correlates the test efficiency for simultaneous detection of normal and abnormal gestations with progesterone concentrations. Peak efficiency occurred when progesterone concentrations ranged from 9 to 14 ng/ml. The test efficiency ranged from 79.7% to 80.4% in this range of progesterone.

Comment

Human chorionic gonadotropin (hCG)[8-13] and ultrasonographic monitoring[14] of early pregnancy usually require several days of observation before a confident diagnosis can be determined. Furthermore, ectopic gestations may not produce sufficient quantities of hCG to raise the serum concentration of hCG to the discriminatory ultrasonographic range before rupture or tubal injury occurs. Because of these limitations, other rapid monitors or early gestational normalcy have been sought.

Progesterone monitoring of early gestations has been studied as an alternative to serial hCG monitoring by several authors.[2-5] Only a single measurement is required compared with the serial observations required for hCG concentrations. Unfortunately, most reports describing the use of progesterone have established cutoff points empirically and have not sought the "best test" of their analysis. Statistical tools that allow informed decisions concerning the best test include the receiver-operator characteristic curve, the predictive value of the test, and test efficiency.[6] All offer simple computational solutions to the problem faced by physicians who must determine the best test for a clinical condition. They allow us to maximize the detection of the condition of interest and minimize erroneous classifications, and they are ideally suited for the problem of the best test analysis here.

The advantage of the receiver-operator characteristic curve is its ability to determine the best test to diagnose a condition. Fig. 2 illustrates two important points concerning the progesterone cutoff points. First, as the progesterone concentration increases, both the sensitivity and false-positive rate increase. Second, any progesterone concentration can be selected as a discriminatory cutoff, but the best cutoff is that associated with highest sensitivity and lowest false-positive rate. In our data, receiver-operator characteristic curve analysis in-

dicated that a progesterone concentration <10 ng/ml is the optimal discriminatory test. False-positive rates are <5% (0% to 3.9%) at this concentration, and the maximum sensitivity is 59%. Higher sensitivities can be obtained at higher progesterone concentrations, but the false-positive rate increases dramatically as well. Additionally, the receiver-operator characteristic curve demonstrates that progesterone monitoring of early gestations cannot distinguish between ectopic pregnancies and abortions.

At the derived best cutoff point (10 ng/ml), the predictive value, rather than the sensitivity, is the best indicator of how well the test will perform. The abnormal test result of abnormal gestations was >90% (92% to 100%) when the progesterone level was <10 ng/ml. This suggests that when the discriminatory progesterone concentration is 10 ng/ml, this test would correctly identify at least 90% of all abnormal gestations. Unfortunately, the normal test result is unable to determine gestational normalcy. The probability of a normal gestation is not >80% until the progesterone level is ≥18 ng/ml. Thus caution must be exercised by the clinician to not overinterpret the significance of a high serum progesterone concentration. Further analysis of the ability of the normal test result to exclude ectopic pregnancies and detect normal gestations is required.

Test efficiency analysis indicated that simultaneous detection of abnormal and normal conditions was maximal when the progesterone cutoff ranged from 9 to 14 ng/ml. This reassures us that the cutoff point of 10 ng/ml derived for the receiver-operator characteristic provides high predictive value and maximum test efficiency.

Potential biases of this study may affect our analysis. First, the optimal progesterone cutoff point may vary as a function of the specific reagents or assay used to determine serum progesterone concentrations. Second, our study incorporated multiple progesterone measurements on each patient. The average number of observations for each patient in the three groups was not significantly different (normal 1.8, abortion 1.6, ectopic 2.0, $p = 0.199$). This potential bias could be eliminated by increasing the number of patients and using only one data point per patient. Another potential bias is the relatively high proportion of ectopic pregnancies identified in this study group. We purposely excluded abortion and ectopic patients who had symptoms or an obvious diagnosis. Such patients were managed on an emergency basis without the need to estimate their risk. Additionally, many patients in our practice were excluded because they conceived after follicular stimulation with human menopausal gonadotropins or clomiphene citrate. These preparations significantly increase progesterone concentrations in all three groups (data not shown).

We believe that our analysis is representative of women who conceive spontaneously. It is the first attempt to determine the "best" discriminatory progesterone concentration on the basis of statistical modeling of the test. As our series enlarges and as additional tests are derived, these can be measured against our preliminary data. In this fashion informed decisions concerning the efficacy of new tests and the best cutoff point for progesterone can be compared.

We gratefully acknowledge the skilled technical assistance of Denise M. Daniels and Angela T. Remley, and the editorial assistance of Pamela K. Fryer and Donna White.

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Discussion

DR. THOMAS G. STOVALL, Memphis, Tennessee. Serial quantitative hCG titers in samples taken 48 hours apart have long been the standard endocrinologic

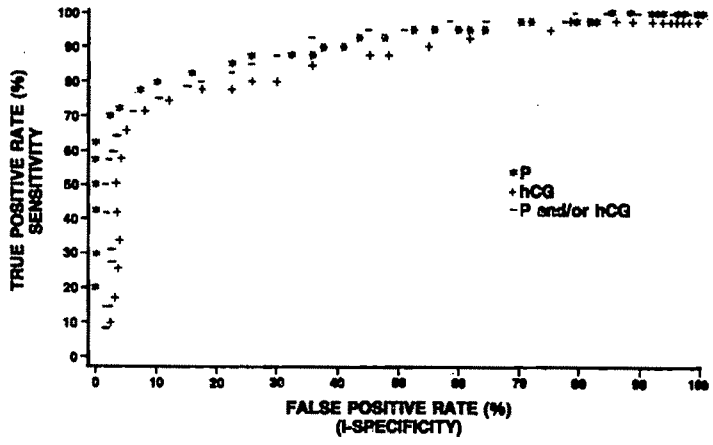


Fig. 1. Receiver-operator characteristic curves for three methods of pregnancy screening. Curve for progesterone *(P)*; *plus signs*, two hCG titers taken 48 hours apart; *minus signs*, progesterone combined with serial hCG titers.

method used to help diagnose ectopic pregnancy. Unfortunately, up to 21% of patients with ectopic pregnancy can have normally rising hCG titers.[1] Also, serial titers require at least two blood samples spaced by 48 hours, thereby delaying diagnosis by ≥2 days.

Cowan and his colleagues have used receiver-operator curves analysis, which graphically represents combinations of comparisons between sensitivity and specificity for a partial test. The better the test for detecting an abnormal condition, the further upward and to the left the curve will be displaced.

They have suggested that the discriminatory progesterone level should be 10 ng/ml; however, as can be seen in Fig. 1, at this level one is unable to determine whether a gestation is normal. The probability that the pregnancy is normal is not >80% until the progesterone concentration ≥18 ng/ml. This is inadequate for clinical practice. To use this test in clinical practice to predict normality, the test needs to be predictive in virtually 100% of cases. Thus, to predict pregnancy normality, the 25 ng/ml cutoff should be used.

At the 1988 American Fertility Society Meeting, our group reported the receiver-operator characteristic curve of 1120 first-trimester patients and compared the sensitivity and specificity of serial hCGs and progesterone at various cutoff levels.[2] The curves from our investigation are shown in Fig. 1, whereas Table I compares the sensitivity and specificity of progesterone at multiple cutoffs alone and in combination with an hCG rise of <66% over a 48-hour period with these data.

Should a single progesterone value replace serial hCG titers for ectopic pregnancy diagnosis? With our data, at ≥25 ng/ml, the sensitivity of a single progesterone level for detecting normal pregnancy is 0.970. The sensitivity rises only 0.022 with the addition of two hCG titers separated by 48 hours. In our series only one additional abnormal pregnancy would have been detected, with no additional ectopic gestations identified. If the screening progesterone level is ≥25 ng/ml, virtually all of these patients have viable intrauterine pregnancies. Only 1% to 1.5% of ectopic pregnancies are associated with progesterone levels of ≥25 ng/ml.

Table I. Comparison of sensitivities and specificities of single serum progesterone measurement at different values, serial hCG titers drawn 48 hours apart, and combination of serum progesterone and hCG titers

Characteristic	*Sensitivity*	*Specificity*
Progesterone <10 ng/ml	0.805	0.982
Progesterone <15 ng/ml	0.887	0.645
Progesterone <25 ng/ml	0.970	0.301
hCG <66% rise	0.887	0.516
Progesterone <10 ng/ml and hCG <66% increase	0.722	0.935
Progesterone <10 ng/ml or hCG <66% increase	0.970	0.473
Progesterone <15 ng/ml and hCG <66% increase	0.797	0.817
Progesterone <15 ng/ml or hCG <66% increase	0.977	0.344
Progesterone <25 ng/ml and hCG <66% increase	0.865	0.688
Progesterone <25 ng/ml or hCG <66% increase	0.992	0.129

The first international reference preparation of hCG was used.

To be of optimal clinical utility in predicting an abnormal pregnancy, any test would have a 100% specificity, but sensitivity would then be sacrificed. This level of specificity is mandatory if patients are to undergo endometrial curettage on the basis of an abnormal test result. We recently reported another series of over 1100 serum progesterone levels in patients with viable pregnancies.[3] The lowest serum progesterone level associated with a viable pregnancy was 5.1 ng/ml, leaving us to choose a cutoff value of 5.0 ng/ml. Therefore, if the serum progesterone value is <5.0 ng/ml, the preg-

nancy is nonviable in 100% of cases and the patient can undergo curettage without fear of interrupting a viable gestation.

On the basis of both our data and the data presented by Cowan et al. I believe the role of serum progesterone in clinical practice appears to be that of a screening tool for both normal and abnormal pregnancies. If the screening progesterone level is ≥25 ng/ml, the patient can be reassured that the pregnancy is viable in >95% of cases; if the progesterone level is <5.0 ng/ml, the pregnancy is considered nonviable. Patients can undergo evacuation of the uterus without fear of interrupting a viable pregnancy. On the other hand, if the serum progesterone level is ≥5 but <25 ng/ml, further testing, including serial hCG titers and transvaginal ultrasonography, is necessary.

This study is an excellent contribution to our body of knowledge concerning the role of serum progesterone screening. I congratulate Dr. Cowan and colleagues on their work.

The following questions are offered for discussion: (1) Do the authors screen all pregnant patients with a single serum progesterone level or only those patients with symptoms or risk factors for ectopic pregnancy? (2) Do the authors feel comfortable basing their results on only 40 normal and 34 abnormal pregnancies, of which only 18 were ectopic pregnancies?

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Dr. Asghar Afsari, West Bloomfield, Michigan. Predicting the well-being of an early pregnancy from one or more maternal serum hormones has been the subject of many investigations. When multiple parameters have been used to better appreciate the diagnostic significance of each, the receiver-operator characteristic curve has been used. It is shown that the serum progesterone level is the single most important predictor of pregnancy viability.[1]

Dr. Cowan and his colleagues have tried to demonstrate the performance of serum progesterone levels for the predictive value and efficiency level at various concentrations. A cut-off point of 10 ng/ml is suggested to be an optimal discriminatory level to differentiate between a normal versus an abnormal pregnancy. However, pragmatically speaking, a sensitivity of 59% as claimed by the authors is not a desirable level to comfortably counsel patients. I agree with the authors that a cut-off between 9 and 14 ng/ml may be a maximum efficiency level for detection of abnormal and normal conditions, although the higher the progesterone level the better the predictive chance to have an intact normal intrauterine pregnancy. Furthermore, the test is 100% predictive of an abnormal pregnancy when the level of serum progesterone is ≤6 ng/ml, as has been demonstrated by the other authors earlier. It is also further apparent from the manuscript that there is always an overlap of serum progesterone concentrations in normal and abnormal pregnancies. The critical level where abnormal and normal test results cross is at a serum progesterone level between 14 and 15 ng/ml, at a predictive value of 79%. Because of this, monitoring of early pregnancies by transvaginal ultrasonography and prompt interpretation of these data and serial serum B-hCG are important. The last two parameters in conjunction with serum progesterone levels will further offer to confirm the differential diagnosis between tubal pregnancy and abortive intrauterine gestation.

As a side remark, Dr. Cowan and his coauthors have made a point that by using the receiver-operator characteristic curve they have been able to avoid an empiric cutoff of the serum progesterone level. The detailed and elaborate monograph by Charles E. Metz clearly explains, "The ROC curve is shown to be a simple, yet complete empirical description of threshold effect, indicating all possible combinations of the relative frequencies of the various kinds of correct and incorrect decisions."[2]

I agree with the authors that an optimal serum progesterone cutoff point would vary depending on the reagents, assay methods, and kits used in various laboratories. Additionally, perhaps this sort of clinical interpretation would require a larger amount of patients.

Finally, I have several questions for the authors. (1) In patients with tubal pregnancies, what was the percentage of rupture? Was a single serum progesterone level as critical as multiple serum measurements and has there been any time delay to reach a diagnosis as a result of this phenomemon? (2) Did the estimated gestational age of pregnancy from LMP influence the overall serum progesterone concentration? (3) What was the outcome of normal pregnancies whose serum progesterone concentration was <15 and ≥10 ng/ml? Was there any treatment rendered and what was the percentage of babies taken home?

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Dr. C. Brandon Chenault, San Antonio, Texas. I wonder about the variations in laboratory in terms of interpreting these parameters.

Dr. Cowan (Closing). Dr. Stovall I think has focused on the controversy of serum progesterone measure-

ments in trying to determine the presence or absence of abnormal pregnancies. We do not use this test to make that determination. We use this test to make a determination of a risk. I think that if you're going to use the test to say whether the patient has an abnormal or a normal pregnancy, you need to use parameters that give you 100% predictability.

There are two important things that you can derive from the receiver-operator characteristic. One is information about what that value means for the application to your clinical population, and the second is other tests from other groups. Other studies or new information that you may put into your data base can be applied with the same type of interpretation.

This analysis was a retrospective analysis. We screen our patients with hCG doubling and ultrasonography, but we retrospectively analyzed the progesterone once we knew what their diagnosis was. We are confident with the small numbers; they are consistent with Dr. Stovall's larger numbers. Our confidence is derived from the statistical ability to compare information from new studies with the ROC analysis.

To address Dr. Stovall's and Dr. Sokol's comments, I agree that there still needs to be the acid test. We must move to the clinical arena and ask the question, "Can we use serum progesterone to screen or detect the abnormal condition that we can retrospectively analyze in a computational model?" Dr. Stovall has published two reports that have addressed the application of that type of testing.

To answer Dr. Afsari's questions, none of our patients had ruptured ectopic pregnancies. All were diagnosed early in the course of their condition with hCG monitoring and ultrasonography. There were no delays based on serum progesterone measurements, and all patients received treatment with either laparoscopic surgery or methotrexate on the basis of the patient's preference.

The time from the LMP does influence the serum progesterone concentration in both normal and abnormal pregnancies. A linear regression model of progesterone concentrations over time shows that time negatively influences the serum progesterone level in the first 49 days of pregnancy. Dr. Afsari asked about the pregnancy outcome of the patients with normal pregnancies and progesterone values <15 or <10 ng/ml. We provided no treatment for these patients. They did not receive supplemental progesterone. All of these were normal pregnancies, and the rate of babies taken home was 100% for this group of patients.

Dr. Chenault asked about laboratory variations. This is a very important consideration. In comparing progesterone values, each laboratory or group of investigators will need to derive their particular progesterone cutoff. With receiver-operator characteristic analysis the aspects of each particular assay and its variability can be obviated by the ability to return to the receiver-operator characteristic analysis and simply determine how a new population compared with, for example, our population or Dr. Stovall's population.

And last, for Dr. Sokol, I agree. It's time to take this type of information to the clinical environment to see if we can do as well as we think we might be able to do.

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Prevalence of out-of-phase endometrial biopsy specimens

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Indianapolis, Indiana

OBJECTIVE: We attempted to determine the prevalence of out-of-phase endometrial biopsy specimens among fertile and infertile women and women with recurrent pregnancy loss, histologic dating of biopsies was compared with four reference points for expected ovulation. These reference points included last menstrual period, next menstrual period, luteinizing hormone testing, and ultrasonographic documentation of ovulation.
STUDY DESIGN: Four hundred eight-five endometrial biopsies were performed 7 days after documented ovulation-based ultrasonographic evidence for follicle collapse. The histologic dating was referenced to the last menstrual period, next menstrual period, and ultrasonographic documentation of ovulation. One hundred thirty-two of these women also performed urinary luteinizing hormone surge testing before ovulation and serum progesterone determinations. A comparison of the prevalence of out-of-phase biopsy specimens among groups was determined with the χ^2 test and Fisher's exact test.
RESULTS: The prevalence of out-of-phase endometrial biopsy specimens ranged from 42% when last menstrual period was ued to 26% with next menstrual period, to 21% with luteinizing hormone testing, and to 4% with ultrasonographic documentation of ovulation. Serum progesterone values among women with a diagnosis of out-of-phase biopsy specimens by any of the reference dates progesterone were similar to those with in-phase biopsy specimens.
CONCLUSION: The accuracy of histologic endometrial dating was best determined by ultrasonographic monitoring rather than by last menstrual period, next menstrual period, or luteinizing hormone testing in infertile populations and in those with recurrent pregnancy loss. Additionally, because no significant difference in out-of-phase biopsy specimens exists between fertile and infertile patients and recurrent pregnancy loss, those with the role of this procedure is called into question. (Am J Obstet Gynecol 1992;166:1738-46.)

Key words: Out-of-phase biopsy specimens, ovulation, endometrial biopsies, infertility, recurrent pregnancy loss

Luteal phase defect was initially defined as a defective corpus luteum with insufficient progesterone production, whether in amount or duration.[1] Since its first description, there has been a lack of consensus as to the role of luteal phase defect as a cause of reproductive failure. Disagreement has primarily been a result of a lack of controlled studies to determine whether correction of the luteal phase defect is of value in treating patients with reproductive problems. Accurate conclusions about treatment must be preceded by accurate diagnosis of the defect. Diagnosis by serum progesterone level, menstrual history, and shortened luteal phase have been inadequate. The accepted standard of diagnosis remains an endometrial biopsy with histologic dating and the criteria established by Noyes et al.[2] Traditionally, histopathologic dating has been compared to onset of next menstrual period; dating is started at day 28 and counted backward.[1, 3] The day of ovulation is considered to be day 14 in a normal cycle. The conventional diagnosis of luteal phase defect has been made by demonstrating a discrepancy between histologic dating and menstrual history by 2 days.[1, 3]

More recently, measurement of luteinizing hormone (LH) or demonstration of ovarian follicular rupture with ultrasonography has allowed a more objective documentation of ovulation.[4] In a recent study,[5] when histologic dating of the endometrium was correlated with postovulatory duration as determined by daily transvaginal ultrasonographic scanning, serum LH determination, basal body temperature, and subtraction of 14 days from onset of next menstrual period in 13 fertile women, the accuracy of dating was significantly better with days from ovulation determined by ultrasonography.

The purpose of this study was to compare the frequency with which the diagnosis of out-of-phase biopsy specimens is made on the basis of dating of the endometrial biopsy specimen from the onset of the last and the next menstrual cycles and from the time of ovulation documented by urinary LH testing and serial ultrasonographic examinations. The prevalences of out-of-phase biopsy specimens with each of these ref-

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6/6/37133

erence points was compared among infertile women, women experiencing recurrent pregnancy loss, and fertile women. These results are also compared with midluteal phase serum progesterone concentrations. The results of this study will be useful in managing cases in which patients are being evaluated for reproductive failures.

Material and methods

Patients. Four hundred eighty-five women were studied; of these, 340 were infertile, 115 had experienced recurrent pregnancy loss, and 30 were fertile.

Infertility was defined as the inability to achieve desired pregnancy after 1 year of unprotected intercourse. The evaluation of couples presenting with infertility consisted of documentation of ovulation by means of urinary LH testing, monitoring of folliculogenesis with serial ultrasonographic examinations, evaluation of luteal phase serum progesterone concentrations, and endometrial biopsies. Sperm availability was documented by semen analysis and the hamster egg penetration assay. Male factor as a cause of infertility was diagnosed if the sperm concentration was <20 million spermatozoa per milliliter, motility and/or normal morphologic characteristics were <50%, and/or the hamster egg penetration assay revealed <12% penetration. Tubal factor as a cause of infertility was assessed by hysterosalpingography and pelvioscopy. To be assigned a diagnosis of tubal factor as a cause of infertility, a woman had to have tubal occlusion documented by hysterosalpingography and pelvioscopy, peritubal adhesions displayed at the time of pelvioscopy, or a history of having undergone a previous tuboplasty. The diagnosis of endometriosis was made by demonstration of endometrial implants within the peritoneal cavity at the time of pelvioscopy. Unexplained infertility was diagnosed if the evaluation yielded no discernible cause.

Recurrent pregnancy loss was defined as two or more consecutive spontaneous abortions. Evaluation of couples with recurrent pregnancy loss included chromosome analysis of both partners, hysterosalpingography and hysteroscopy of the female partner, luteal phase serum progesterone concentration and endometrial biopsies; autoantibody testing, including tests for antinuclear antibodies and antiphospholipid antibodies (e.g., anticardiolipin and antiphosphatidylserine antibodies), activated partial thromboplastin time, assays for the presence of maternal antipaternal lymphocytotoxic antibodies[6]; mixed lymphocyte culture; and human leukocyte antigen typing of both partners.

The control group was composed of 30 volunteer women from the Indianapolis community. Complete medical and obstetric histories were obtained from each woman. A fertile control was defined as a woman who had had two or more successful pregnancies and who was able to conceive a desired pregnancy within 1 year of unprotected intercourse. Volunteers with three or fewer pregnancies have had no spontaneous abortions and also must have had at least two children. Volunteers with four or more pregnancies may have had one spontaneous abortion. However, the most recent pregnancy must have been normal, and these women must have been able to meet the other criteria for women with three or fewer pregnancies.

Menstrual history. Each woman had her menstrual history, including the onset of her last menstrual period (LMP) and the next menstrual period during the monitored cycle, recorded. When the LMP was used as a reference point by which to date the endometrial biopsy specimen, ovulation on day 14 of a 28-day menstrual cycle was assumed. When the next menstrual period was used as a reference of the day of endometrial biopsy, a 14-day luteal phase (and hence ovulation) was assumed to have occurred 14 days before the onset of the next menstrual period.

Endometrial biopsy. All women had an endometrial biopsy performed during the luteal phase of the menstrual cycle 7 to 10 days after documented ovulation on the basis of ultrasonography. Biopsy specimens were obtained with a Novak curette, and the tissue was fixed in a 10% neutral buffered formalin solution and embedded in paraffin blocks. Hematoxylin and eosin–stained sections were examined and dated according to the criteria of Noyes et al.[2] The histologic dating was compared with the cycle day of the biopsy according to the onset of the LMP, the onset of the next menstrual period, the urine LH level, and ovulation on the basis of ultrasonographic monitoring. Out-of-phase biopsy specimens were diagnosed if the histologic appearance of the endometrial biopsy specimen was >2 days out of phase from the day on which the biopsy was performed.

LH testing. LH testing was done by qualitative means using the Q Test ovulation kit (Clay Adams, Parsippany, N.J.). This is a monoclonal antibody enzyme immunoassay dipstick designed to visually detect human LH in urine specimens. Sensitivity of the Q Test is 97% relative to reference radioimmunoassay ≥60 mIU/ml) for urine samples. Patients were instructed to collect first-morning urine specimens daily from cycle day 10 until an LH surge was detected. The dipsticks were blotted and included in the patient's medical record. Ovulation was assumed to occur within 24 hours of LH surge.

Ultrasonographic monitoring of ovulation. Ultrasonographic examination was begun on days 8 to 10 of the menstrual cycle and continued every 2 to 3 days until follicle rupture was documented. The criteria for ultrasonographic evidence of ovulation have been described by Hill et al.[4]: The dominant follicle shows loss

Table I. Clinical characteristics of 485 women participating in endometrial biopsy study

Women	Age (yr)		Gravidity	
	Mean	Range	Mean	Range
Infertile	32	21-42	0.9	0-5
Recurrent spontaneous abortion	32	21-42	4.0	2-11
Fertile	31	22-37	3.0	2-5

Table II. Frequency of causes of infertility diagnosed in 340 couples

Cause of infertility	No.	%
Tubal factor	120	35
Unexplained	106	31
Male factor	76	23
Endometriosis	38	11
TOTAL	340	100%

of clear demarcation of its walls, there is a decrease in follicular size, and intrafollicular echoes occur. In addition, increased volume of fluid in the cul-de-sac suggested ovulation.

Serum progesterone determinations. Blood was drawn from each woman at the time of endometrial biopsy. Each blood specimen was evaluated for serum progesterone concentration with a radioimmunoassay (Diagnostic Products Corporation, Los Angeles).

Comparative analysis. The prevalence of diagnosis of out-of-phase biopsy specimens on the basis of histologic dating of the endometrium from the LMP and the next menstrual period was compared with the frequency of diagnosis of out-of-phase biopsy specimens on the basis of ovulation documented by LH testing and/or ultrasonographic monitoring. The χ^2 test and Fisher's exact test were used. The prevalence of diagnosis of luteal phase defect on the basis of mean midluteal phase progesterone concentration for each group was determined by Fisher's exact test. These comparisons were made among infertile women, women experiencing recurrent pregnancy loss, and fertile control women.

Results

Patients. Table I summarizes the clinical characteristics of the women participating in the study. The mean age of the infertile women was 32 years (range 21 to 42 years) in women with recurrent pregnancy loss, it was 32 years (range 21 to 42 years), and in fertile women it was 31 years (range 22 to 37 years). The mean gravidity among women with recurrent spontaneous abortion was 4 (range 2 to 11); among fertile control women, 3 (range 2 to 5); and among infertile women, 0.9 (range 0 to 5).

The frequency of diagnosis of infertility is summarized in Table II. Of the 340 infertile couples studied, 120 (35%) had the diagnosis of tubal factor as a cause of infertility; 106 (31%), unexplained infertility, 76 (23%), male factor, and 38 (11%), endometriosis. Those women who had a diagnosis of ovulatory factor as a cause of infertility were not included in this study.

Prevalence of out-of-phase biopsy specimens. The prevalence of out-of-phase endometrial biopsy specimens among infertile women, women experiencing recurrent pregnancy loss and fertile women is summarized in Figs. 1 to 4.

Among 340 infertile women, 146 (43%), 92 (27%), and 12 (3.5%) displayed histologic evidence of out-of-phase endometrial biopsy specimens when the LMP, the next menstrual period, and ultrasonographic results, respectively, were used as reference points for ovulation (Fig. 1). Urinary LH testing was performed by 93 of the 340 infertile women, of whom 20 (22%) had histologic evidence of out-of-phase endometrial biopsy specimens. χ^2 analysis revealed that significantly fewer out-of-phase biopsy specimens were diagnosed when ultrasonography was used as a reference for histologic dating rather than the LMP, the next menstrual period, or LH testing ($p < 0.05$), but no significant difference was noted between results from the use of the next menstrual period and LH testing (Fig. 1).

Fig. 2 demonstrates the prevalence of out-of-phase endometrial biopsy specimens among 115 women experiencing recurrent spontaneous abortions. Among these 115 women, 45 (38%), 28 (24%), and 6 (5%) had histologic evidence of out-of-phase endometrial biopsy specimens when LMP, the next menstrual period, and ultrasonographic results, respectively, were used as reference points for ovulation. When urinary LH testing was performed by 24 women, 5 (20%) had histologic evidence of out-of-phase endometrial biopsy specimens. Again, χ^2 analysis revealed significantly fewer out-of-phase biopsy specimens when ultrasonography was used as the reference for histologic dating rather than the LMP, the next menstrual period, or LH testing ($p < 0.05$).

Among 30 fertile women, 15 (50%), 9 (32%), and 3 (10%) had histologic evidence of out-of-phase endometrial biopsy specimens when the LMP, the next menstrual period, and ultrasonographic results, respectively, were used as reference points for ovulation (Fig. 3). Fifteen of the 30 fertile control women performed urinary LH testing as a reference for ovulation. As a result of urinary LH testing, 4 of 15 (26%) demonstrated out-of-phase endometrial biopsy specimens. Among fertile controls, Fisher's exact test demonstrated significantly fewer out-of-phase biopsy speci-

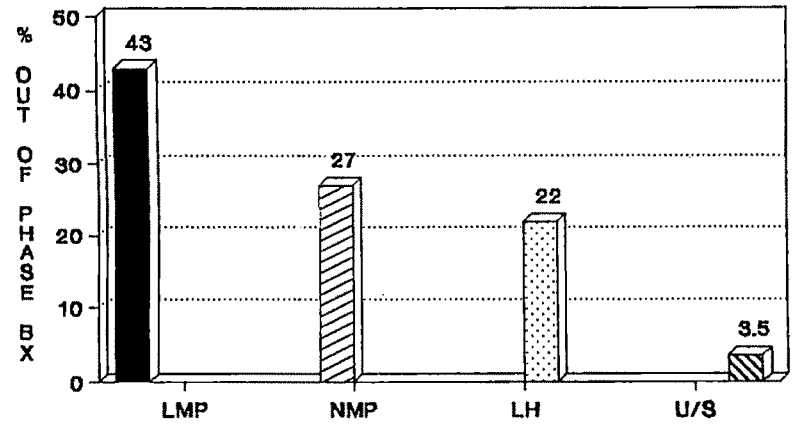


Fig. 1. Prevalence of out-of-phase endometrial biopsy specimens among 340 infertile women; biopsies are on basis of onset of LMP, next menstrual period *(NMP)*, urinary LH testing, and documentation of follicle rupture by ultrasonographic examination *(U/S)*.

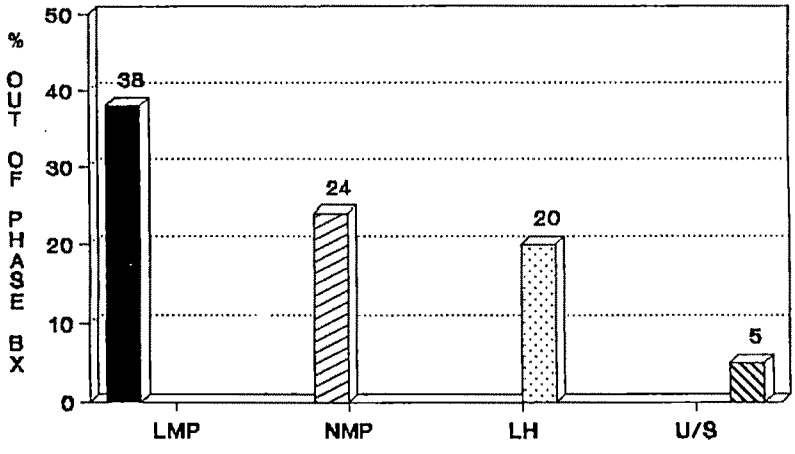


Fig. 2. Prevalence of out-of-phase endometrial biopsy specimens among 115 women with history of recurrent spontaneous abortion on basis of onset of LMP, next menstrual period *(NMP)*, urinary LH testing, and documentation of follicle rupture by ultrasonographic examination *(U/S)*.

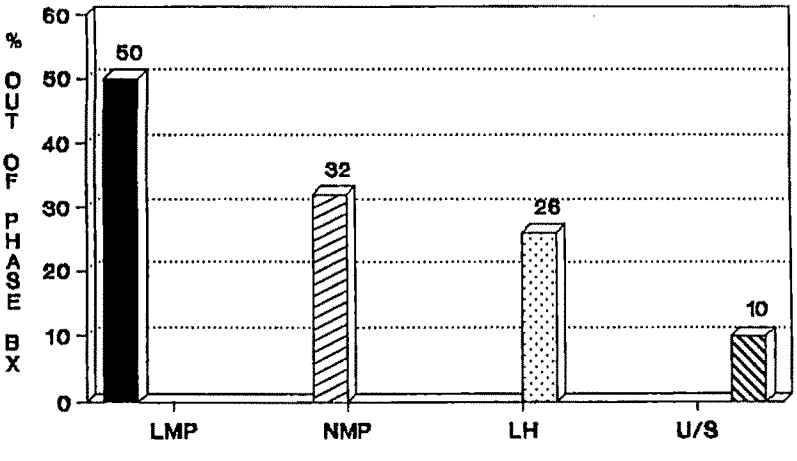


Fig. 3. Prevalence of out-of-phase endometrial biopsy specimens among 30 fertile women on basis of onset of LMP, next menstrual period *(NMP)*, urinary testing, and documentation of follicle rupture by ultrasonography examination *(U/S)*.

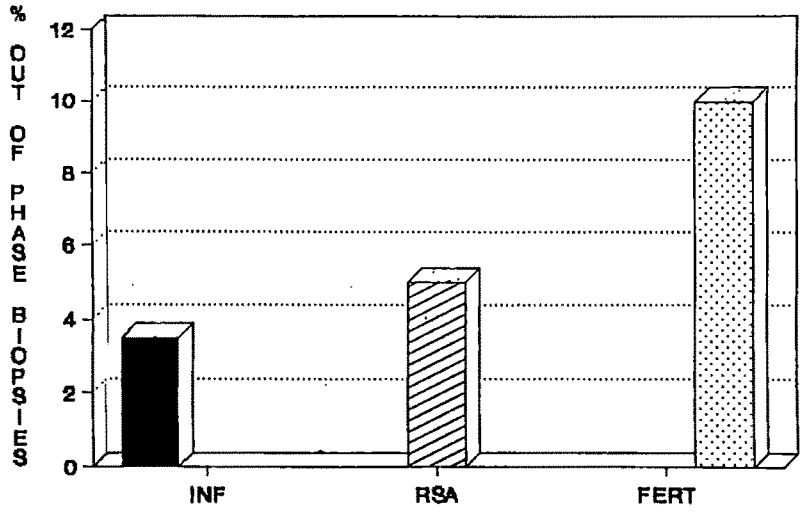


Fig. 4. Prevalence of out-of-phase endometrial biopsy specimens among 340 infertile women *INF*, 115 women experiencing recurrent spontaneous abortion *(RSA)*, and 30 fertile women *(FERT)* when ultrasonographic documentation of ovulation was used to compare dating of endometrial biopsy specimens.

Table III. Prevalence of out-of-phase endometrial biopsy specimens among infertile women by specific cause of infertility

	Prevalence of out-of-phase biopsy specimens							
	LMP		*NMP*		*LH*		*US*	
Cause of infertility	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
Explained	100	42	71	30	14	20	9	4
Tubal factor	54	45	38	32	7	21	3	2
Male factor	36	47	27	36	7	25	5	6
Endometriosis	10	26	6	16	0	0	1	2
Unexplained	46	43	21	20	6	24	3	3
TOTAL	146	43	92	27	20	22	12	4
Significance					$p < 0.05$*		$p < 0.05$*	

NMP, Next menstrual period; *US*, ultrasonographic testing.
*Significantly fewer out-of-phase biopsy specimens were observed when either LH or ultrasonographic testing was used as the reference for histologic dating of biopsy specimens.

mens when ultrasonography was used as a reference for histologic dating rather than the LMP ($p < 0.05$).

Fig. 4 summarizes the prevalence of out-of-phase endometrial biopsy specimen among 340 infertile women, 115 women experiencing recurrent spontaneous abortion, and 30 fertile women when ultrasonographic documentation of ovulation was used as a point of reference in comparison of the histologic findings of the endometrial biopsy specimen. The prevalence of out-of-phase endometrial biopsy specimens was 3% in infertile women, 5% in women experiencing recurrent spontaneous abortion, and 10% in fertile women. A χ^2 analysis revealed no significant difference among these groups ($p > 0.05$).

Further analysis of the prevalence of out-of-phase endometrial biopsy specimens by specific cause of infertility is shown in Table III. The prevalence of out-of-phase endometrial biopsy specimens was 4% among women with an explained cause of infertility and 3% among those with an unexplained cause when ultrasonographic documentation of ovulation was used as a reference for comparison of the histologic findings of the endometrial biopsy (Table III).

Serum progesterone determinations. Mean serum midluteal progesterone concentrations from 93 infertile women, 24 women experiencing recurrent pregnancy loss, and 15 fertile women are presented in Table IV.

When mean serum progesterone concentrations from infertile women with out-of-phase endometrial biopsy specimens were compared with serum progesterone values from infertile women with in-phase endometrial biopsy specimens, no differences were observed. Sixty-eight of 93 infertile patients had an explainable cause of infertility (i.e., tubal factor, male factor, endometriosis). Of these 68 explained infertile patients, 8 had an out-of-phase endometrial biopsy specimen and 60 had in-phase biopsy specimens when ultrasonographic results were used to determine ovulation. The mean serum progesterone concentrations were 16.0 and 11.7 ng/ml, respectively. No significant difference exists between these serum progesterone values ($p > 0.05$). The causes of infertility in 25 of the 93 infertile patients were unexplained. Two of 25 patients with unexplained infertility had out-of-phase biopsy specimens with a mean serum progesterone level of 14.7 ng/ml. Twenty-three of 25 patients with unexplained infertility had in-phase biopsy specimens with

Table IV. Comparison of mean serum midluteal progesterone concentrations and prevalence of out-of-phase endometrial biopsy specimens among women with various causes of infertility, women with a history of recurrent pregnancy loss, and fertile control women

Endometrial biopsy specimens	*No.*	*Mean serum progesterone (ng/ml)*		*Significance**
		Out of phase	*In phase*	
Infertility				
Explained	68	16.0 (8)†	11.7 (60)	$p > 0.05$
Tubal factor		11.8	11.3	
Male factor		13.7	12.1	
Endometriosis		37.6	13.6	
Unexplained	25	14.7 (2)	12.5 (23)	$p > 0.05$
Recurrent pregnancy loss	24	2.4 (1)	13.8 (23)	$p > 0.05$
Fertile controls	15	10.6 (2)	10.3 (13)	$p > 0.05$
Total no. of patients	132	13	119	

*Fisher's exact test demonstrates no significant difference in mean serum midluteal progesterone values between in-phase and out-of-phase endometrial biopsy specimens for patients with explained infertility, unexplained infertility, or recurrent pregnancy loss when compared with fertile control patients.

†Numbers in parentheses are numbers of biopsies that represent this mean midluteal serum progesterone concentration.

a mean progesterone value of 12.5 ng/ml. No significant difference exists between these mean serum progesterone values ($p > 0.05$) by Fisher's exact test. Among 24 women experiencing recurrent pregnancy loss, 1 had an out-of-phase endometrial biopsy specimen and had a serum progesterone concentration of 2.4 ng/ml. Twenty-three of 24 patients had in-phase biopsy specimens with a mean serum progesterone level of 13.8 ng/ml. Again, no significant difference exists between mean serum progesterone levels ($p > 0.05$) by Fisher's exact test. Fifteen of 30 fertile control patients were evaluated for a serum progesterone concentration 7 to 10 days after documented ovulation. Two of 15 fertile control women had out-of-phase biopsy specimens with a mean progesterone concentration of 10.6 ng/ml. The remaining 13 women had in-phase endometrial biopsy specimens for a mean progesterone value of 10.3 ng/ml. No significant difference existed here, either ($p > 0.05$). Collectively, 132 women had midluteal phase progesterone concentrations determined, and 13 (10%) displayed out-of-phase endometrial biopsy specimens; 119 (90%) had in-phase endometrial biopsy specimens. No correlation between midluteal phase progesterone concentrations and out-of-phase endometrial biopsy specimens was observed.

Comment

Analysis of results from this study indicate that the frequency of diagnosis of luteal phase defect among women varies markedly as a result of the point of reference used to date the endometrial biopsy specimen. Because of the variance in the length of the follicular phase of the menstrual cycle, dating the endometrial biopsy specimen with reference to the onset of the next menstrual period has been recommended[5] and has become a standard of practice. Although dating the endometrial biopsy specimen by means of the onset of the next menstrual period diminished the frequency of diagnosis of out-of-phase biopsy specimens among couples experiencing infertility compared with the results of dating by means of the onset of the LMP, this accepted method yielded a much higher prevalence of out-of-phase biopsy specimens than did dating according to documented ovulation by ultrasonography.

Our findings confirm observations by Shoupe et al.,[5] who found that the accuracy of histologic endometrial dating was significantly better when ovulation was determined by ultrasonographic monitoring rather than by onset of the next menstrual period, the basal body temperature, or the serum LH value. The prevalence of diagnosis of out-of-phase biopsy specimens fell from 27% to 3.5% among infertile women and from 38% to 5% among women experiencing recurrent spontaneous abortion when ultrasonography, rather than the onset of the next menstrual period was used to document ovulation.

The prevalence of diagnosis of luteal phase defect among infertile patients has been estimated to be 5%,[7-9] which is consistent with the prevalence of out-of-phase biopsy specimens in the current study of couples with unexplained infertility when ovulation documented by ultrasonographic examination was used to date the endometrial biopsy specimen. In contrast, an inadequate luteal phase has accounted for 23% to 67%[10-14] of diagnosed causes of recurrent spontaneous abortions, which is consistent with the results presented here when menstrual dates were used to interpret the endometrial biopsy specimen. However, these figures are substantially higher than those observed when documented ovulation was used to date the endometrial

biopsy specimen. In an evaluation of 195 Norwegian couples with a history of recurrent spontaneous abortion, 5% showed evidence of luteal phase defect.[15] Although an argument has been advanced that progesterone deficiency must be corrected before implantation to prevent recurrent abortion,[1, 10, 16] no placebo-controlled trial has proved this hypothesis. The data presented here suggest that out-of-phase endometrial biopsy specimens and possibly luteal phase defect are not common causes of recurrent pregnancy loss.

In spite of the modality used, the prevalence of diagnosis of out-of-phase endometrial biopsy specimens was as high or higher in the fertile control population when compared with two populations experiencing reproductive failures. The prevalence of out-of-phase biopsy specimens among fertile women was 10% when ultrasonography was used to document ovulation. Others have documented a high prevalence of luteal phase defect among normally cycling women.[5, 17-19] A prevalence of luteal phase defect of 23% has been reported in women with completely normal follicular and periovulatory phases.[18] Implantation is the only gold standard for endometrial adequacy. Endometrial biopsy specimens taken during conceptional cycles were diagnosed as showing luteal phase defect in 23%.[20]

An explanation for the out-of-phase endometrial biopsy specimen in normal women is the inaccuracy of the histologic dating of the endometrium.[21] The accuracy of an endometrial biopsy specimen in the diagnosis of luteal phase defect has been reported to be 25% to 35%.[21, 22] This low accuracy rate has been attributed to improper acquisition of tissue, variability in the method of documentation of ovulation, and reproducibility of reading of the endometrial biopsy specimen by various pathologists. In their original dating criteria Noyes et al.[2] commented that they were "somewhat arbitrary" and that their method represented "sequential phases rather than actual days of the cycle." When these investigators analyzed the accuracy of dating in 300 women in the luteal phase, only 14% of these women menstruated on the day predicted (12% later and 74% earlier than expected). The error ranged from 12 days early to 8 days late; the mean (±SD) was 1.81 ± 2.33 days. The authors concluded that dating is a "better gauge of progesterone effect than it is a prediction of onset of menses."[2]

Subsequent data supported the conclusions of the early study[5] that the endometrial biopsy specimen more accurately represents a progesterone effect than a specific day in the menstrual cycle The diagnosis of luteal phase defect has been made by endometrial biopsy because this method of diagnosis has been thought to reflect the total effect of the endocrine events of the menstrual cycle.[10] Midluteal phase progesterone concentrations have been reported to be inaccurate in diagnosing luteal phase defect because of sporadic fluctuations in secretion of progesterone by the corpus luteum and because many investigations have demonstrated a lack of correlation between endometrial histologic characteristics and peak serum progesterone levels.[21, 23]

In this study no correlation between midluteal phase progesterone concentrations and out-of-phase endometrial biopsy specimens was observed; this confirms the previous reports.[23, 24] However, analysis of the data suggests that out-of-phase endometrial biopsy specimens are not correlated with reproductive performance. Therefore midluteal phase serum progesterone concentration may be as accurate as endometrial biopsy in defining postovulatory corpus luteum function. Although the corpus luteum is important for successful reproduction in mammalian species, the diagnostic methods that pertain to the effects of functional defects on reproductive performance have been questioned.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

Dr. E. James Aiman, Milwaukee, Wisconsin. "Out-of-phase endometrial biopsy specimens are not correlated with reproductive performance." This quotation from the authors' manuscript serves as their hypothesis, their null hypothesis in statistical terms. To accept or reject this hypothesis, the authors obtained one endometrial biopsy specimen from each of 485 women, 340 of these women were infertile, 115 had had repeated pregnancy losses, and 30 were fertile women who served as control subjects. To draw conclusions regarding their stated hypothesis, the authors correlated their histologic findings from this single midluteal endometrial biopsy specimen with five measures of ovulation: each woman's last menses, her next menses, ultrasonographic evidence of ovulation, urinary LH testing, and midluteal serum progesterone concentrations. They used a standard definition of out-of-phase endometrium: 2 days.

To decide whether the authors have performed a study that allows them to accept their stated hypothesis, I will use a metaphor offered by Peterson and Kleinbaum.[1] This is a metaphor of a marksman's target. Precision is the proximity of the data to the bull's-eye. Validity deals with whether the appropriate data are aimed at the right target. By including infertile women, women with repeated pregnancy losses, and a fertile group of women as controls, the authors have three targets that are appropriate together. In the correlation of endometrial histologic factors with five measures of ovulation in these three groups of women the authors have performed a valid study, although the number of fertile women as control subjects (30) is a much smaller sample than those in the other two groups.

It is the precision that weakens the ability of the authors to accept their hypothesis that out-of-phase endometrial biopsy specimens are not correlated with reproductive performance. First, it is somewhat curious that there is an apparent single etiologic factor in all 340 infertile couples, whereas 10% to 20% would be expected to have two or more apparent impediments to fertility. This suggests a selection bias, or perhaps an editorial decision by the authors to categorize these women according to the most prominent cause of infertility.

Precision in the interpretation of the endometrial biopsy specimens may be a significant source of error in any study of endometrial dating. The authors do not state in their manuscript who interpreted the endometrial biopsy specimens whether a single person interpreted all endometrial biopsy specimens, or if the interpreter was blinded to the clinical diagnosis. The precision of interpretation could be tested and probably improved if each biopsy were read a second time by the same interpreter, if that person were unaware of his or her initial interpretation. The precision of interpretation could be tested and probably improved if each biopsy were interpreted by a second person. Scott et al.[2] reported that the diagnosis of in-phase or out-of-phase endometrium was changed in 22% of women when the endometrium was dated by a second person.

Also contributing to the imprecision is the authors' definition of an out-of-phase endometrium by a discrepancy of 2 days. In their manuscript the authors do not state how many of the out-of-phase endometria demonstrated a 2-day lag and how many exhibited a 2-day advance of the number of days after ovulation. The inclusion of multiple histologic criteria from both endometrial stroma and glands may add to the imprecision. By means of quantitative histologic techniques, Li et al.[3] observed that women with unexplained infertility had a significant deviation from normal in only 5 of 14 histologic features. These five features were in the glands; none was in the stroma. Thus the imprecision of the interpretation of endometrial histologic factors is a combination of the "noise" of interpreting multiple histologic events plus interobserver variation.

The authors used serial ultrasonographic measurement to determine the day of ovulation. However, the performed ultrasonographic examinations only every 2 to 3 days, which means that they could be off by as many as 3 days. Because the authors used ultrasonographic criteria to determine the day of ovulation, it is not surprising that an out-of-phase endometrium was found much less frequently with ultrasonographic dating as opposed to the date of the last menses, the date of the next menses, urinary LH testing, or midluteal progesterone concentrations. This interval of ultrasonographic determination potentially adds to the imprecision of their data.

The authors obtained a single serum progesterone concentration on the day of the endometrial biopsy, 7 to 10 days after ultrasonographic evidence of ovulation. Obtaining a single measurement over this range of days ignores the expected daily changes in progesterone concentration and also ignores the variations in the

serum progesterone secretion within a specific day. Nonetheless, the authors' finding that serum progesterone concentrations were similar in all groups of women, regardless of diagnosis or endometrial histologic factors, is similar to that reported by Glazener et al.[4] Olive et al.[5] correlated hourly serum progesterone concentrations over 24-hour period with endometrial histologic factors and concluded that neither endometrial biopsy nor serum progesterone concentration alone is sufficient to establish a diagnosis of luteal phase deficiency. Further, they concluded that pooled morning serum progesterone concentrations provided the optimal predictive value for endometrial biopsy results.

From any discussion it may appear that I find little of value in this manuscript. Quite the contrary is true. Peters and coauthors are to be commended for collecting data on a large number of women and for rigorously defining their selection criteria. From their data and the data of others whom they cite in their manuscript, the authors are justified in concluding that luteal phase deficiency is a difficult diagnosis to make, is found in percentage of women with normal reproduction, and therefore is of uncertain significance in women with repeated pregnancy loss or infertility. Because of the design elements in their study that contribute to a decreased precision, the authors can rightfully suggest but have not proved that "out-of-phase endometrial biopsy specimens are not correlated with reproductive performance."

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Dr. M. Wayne Heine, Tucson, Arizona. The title of the report should probably be changed to "The Accuracy of Timing of Ovulation." The authors use the criteria described by Noyes et al.[1] These authors described the criteria as being "somewhat arbitrary." Because of variance in histologic interpretation, one of the authors read all the slides. Their is the gold standard by which luteal phase function is evaluated. Li et al.[2] reported on 63 endometrial biopsy specimens read by the same pathologist on two separate occasions. There was exact agreement only 24% of the time. Those authors also reported that when two cycles in the same patient were evaluated there was agreement only 41% of the time. Here are questions for Dr. Peters: Who did the histologic interpretation? Were any patients evaluated for more than one cycle?

Some of our work on the evaluation of sleep patterns at various times of the menstrual cycle in "always regular women" showed that 15% of these cycles were anovulatory (unpublished observations). There may be considerable cycle-to-cycle variation. Peters et al. found that the "accuracy of dating" was significantly better when ultrasonographic monitoring was used. The histologic criteria described by Noyes et al.[1] used next menstrual period and not the estimated date of ovulation. Peters and associates used ultrasonographic testing every 2 to 3 days. Could this not give up to a 24- to 48-hour variation in histologic interpretation?

The authors of this study also used serum progesterone studies. Were all these obtained at the same time of the day from a single vein puncture?

None of the various methods used to describe the adequacy of the luteal phase really reflect function. As the authors stated, "implantation" is really the gold standard. The function of the corpus luteum is probably the result of follicular development during the follicular phase. Until we have a more "functional" test of adequacy of ovulation, might it not be more accurate to reevaluate the histologic criteria of Noyes et al.[1] and to use more accurate methods of timing ovulation, which would date the endometrium from the time of ovulation until the biopsy is performed? The authors have accomplished a tremendous amount of work and have a tremendous amount of information. They might want to reevaluate the timing of ovulation by ultrasonography and date the endometrium according to the number of days the biopsy was done after ultrasonographic evidence of ovulation.

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Drug use or inadequate prenatal care? Adverse pregnancy outcome in an urban setting

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OBJECTIVE: This study analyzed the relationships between illicit drug use, low birth weight, adequacy of prenatal care, and perinatal mortality.
STUDY DESIGN: Perinatal outcome parameters in 23,926 deliveries between 1983 and 1990 were analyzed. Statistical analysis (χ^2 analysis) was applied for each year of study to separate the effects of inadequate prenatal care from illicit drug use.
RESULTS: Patients with drug use had a two to three times higher incidence of low birth weight and perinatal death. Drug use with more than five prenatal visits had a minimal effect on outcome; drug use with inadequate care was associated with a three times higher incidence of perinatal death and low birth weight.
CONCLUSION: Adequacy of prenatal care is a marker of "social chaos" and affects outcome more than drug use itself. (Am J Obstet Gynecol 1992;166:1747-56.)

Key words: Drug use, prenatal care, perinatal death, low birth weight

The frequency of illicit drug use during pregnancy has increased markedly in the last decade, especially in urban areas.[1-4] Drug use during pregnancy has been reported to be associated with adverse pregnancy outcome.[2, 5] An increase in the incidence of spontaneous abortion, renal and limb anomalies, preterm labor, and abruptio placentae has been associated with cocaine use.[3-8] Illicit drug use has been associated with inadequate prenatal care, low birth weight, increased perinatal mortality, and sudden infant death syndrome.[1, 4, 6, 7]

Illicit drug use during pregnancy in our own urban institution was ascertained in 1989 by an anonymous urine and questionnaire screening of 1000 patients.[9] This survey indicated that in our institution 15.9% of patients delivered used illicit drugs with crack cocaine (66%) the most common illicit drug. A total of 54% of perinatal drug users used multiple drugs and 84% smoked cigarettes and used alcohol.

This study aimed to analyze the trend in illicit drug use during pregnancy over an 8-year period and to analyze the relationships between illicit drug use, low birth weight, adequacy of prenatal care, and perinatal mortality in an urban perinatal center from 1983 to 1990.

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Material and methods

Since 1983 our institution has collected perinatal data in a computerized data base, described previously.[10] A set of data, routinely used for birth certificate information, quality assurance, and clinical research projects, was collected on all births. Patients were accepted for inclusion in this study if their records contained complete information regarding birth weight, race, age, parity, number of prenatal visits, illicit drug use in pregnancy, cause of perinatal mortality, and assignment of preventability of perinatal death. In addition, all records had to indicate the presence or absence of diabetes mellitus, hypertension, a history of preterm birth, tobacco use, alcohol use, and congenital anomalies in the neonate.

Perinatal mortality was defined as fetal or neonatal death after 20 weeks' gestation or with birth weights >500 gm up to 28 days of neonatal life. Low birth weight was defined as birth weight <2500 gm. Potential preventability had been routinely assigned for each perinatal death by perinatal quality assurance criteria developed in our institution and applied since 1980. A perinatal death was deemed preventable if standard prenatal care was thought to have been able to prevent or modify the obstetric events leading to a perinatal death, thereby preventing the death. Drug use, in the context of this article, included illicit drugs only (opiates, marijuana, cocaine, amphetamines, PCP), and alcohol and tobacco use were not included in this category. Statistical testing was done for each year of the study by χ^2 analysis. A value of $p < 0.05$ was considered statistically significant. Multiple regression analysis was unfortunately impossible in our data setup. Wherever

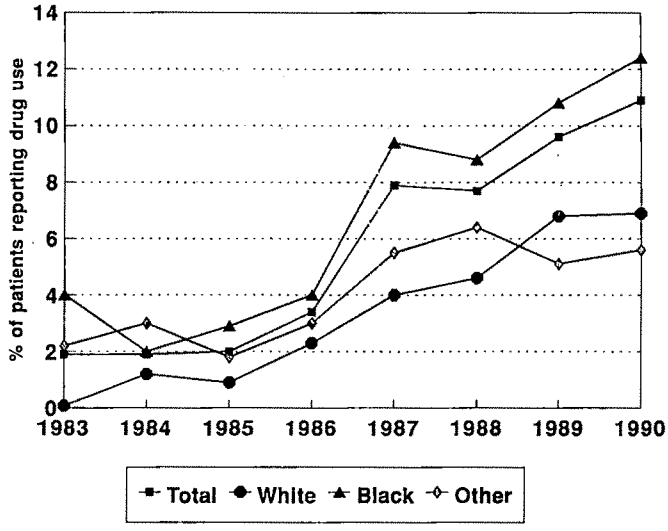


Fig. 1. Illicit drug use by history, 1983 to 1990.

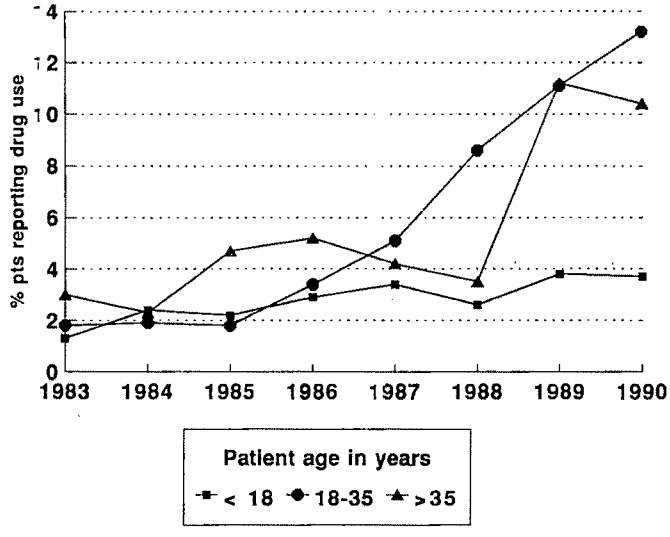


Fig. 2. Drug use in pregnancy by history, 1983 to 1990.

χ^2 analysis was applied, groups compared were checked to be matched for age, parity, hypertensive disease, diabetes mellitus, history of preterm delivery, tobacco use, alcohol use, and congenital anomalies.

Results

Between Jan. 1, 1983, and Dec. 31, 1990, a total of 25,453 patients were delivered at our institution. Excluded from the study were 1527 patients (6%) who had incomplete sets of data. Our analysis therefore is based on 23,926 patients (25.1% white, 70.1% black, and 4.8% of other races). The number of deliveries gradually increased from 2360 in 1983 to 3501 in 1990, an increase exclusively for black patients. Since 1984 several hospitals in our urban area closed and our institution absorbed an indigent black patient load.

The incidence of illicit drug use by patient's history is seen in Fig. 1. A history of illicit drug use during pregnancy was seen in 2% of patients in 1983 and increased to 10.9% in 1990. Increases for all racial groups accelerated around 1986. By 1990 the incidence was 12.2% for black women and 7.1% for white patients.

When illicit drug use was characterized by age group (Fig. 2), it could be seen that drug use in pregnancy is predominantly a problem for women >18 years old. The incidence of illicit drug use for teenagers ranged between 2.1% and 3.9% over the 8 years of study, while for women aged 18 to 35 the incidence increased from 1.8% in 1983 to 13.2% in 1990. In 1990 3.7% of teen-

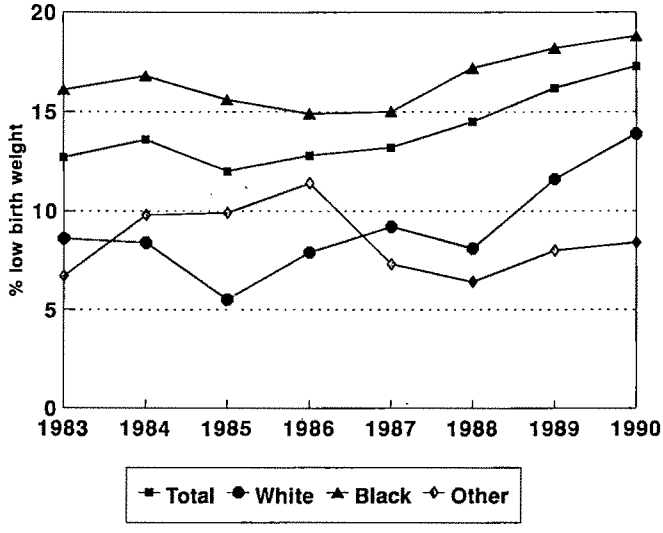


Fig. 3. Low birth weight, 1983 to 1990.

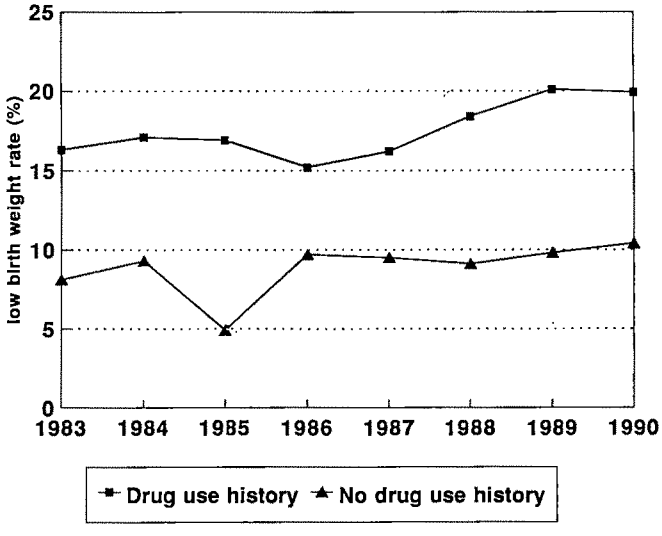


Fig. 4. Drug use and low birth weight, 1983 to 1990.

agers versus 13.2% of women aged 18 to 35 had a history of illicit drug use.

Low-birth-weight rates (Fig. 3) increased after 1987 for all races, reaching 17.3% by 1990, after a relatively stable rate from 12% to 13% from 1983 to 1986. The low-birth-weight rate increased from 5.5% in 1985 to 13.9% for white patients: for black patients it increased from a low of 14.9% in 1986 to 18.8% in 1990. These trends were not similar in all age groups. In fact, the low-birth-weight rate for parturients <18 years old declined from 18.7% in 1983 to 11.4% in 1990, while for patients aged 18 to 35 the rate increased from 12% to 18.1% in the same years.

The relationship between drug use and low birth weight is illustrated in Fig. 4. In each year from 1983 to 1990 the low-birth-weight rate was one and a half to two times higher for patients with a history of illicit drug use compared with patients without drug use. The differences were statistically significant ($p < 0.5$) for each year and for the total data set. No significant differences were found between the two groups in each year for each of the eight potential variables, mentioned under Material and methods. From 1986 to 1990 an increase was seen in low-birth-weight rate for drug users (15.2% to 19.9%) while patients without drug use maintained a rate around 9% to 10%.

Fig. 5 indicates the percentage of patients with five or fewer prenatal visits from 1983 to 1990. Since 1986 the percentage of patients with inadequate care started to rise rapidly, resulting in a fivefold increase for white

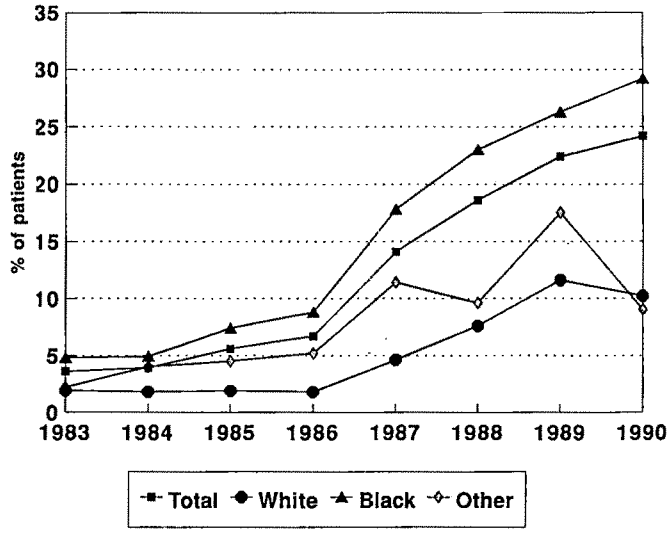


Fig. 5. Five or fewer prenatal visits, 1983 to 1990.

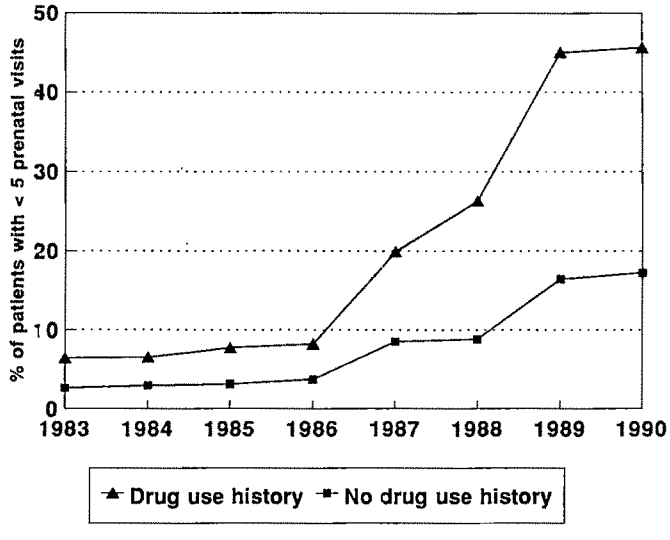


Fig. 6. Drug use and number of prenatal visits, 1983 to 1990.

patients and a sixfold increase for black patients, when 1983 is compared with 1990. Black patients had a two to three times higher likelihood of having five or fewer prenatal visits than did white patients (29.2% vs 10.2% in 1990). While the percentage of patients with five or fewer prenatal visits increased for patients in all groups, the largest increase was seen in women aged 18 to 35. The increase in this age group was from 3.6% in 1983 to 29.5% in 1990, while for teenagers the figures were 2.6% and 15.2% in the respective years.

An analysis of the relationships between drug use and adequacy or inadequacy of prenatal care is seen in Fig. 6. The percentage of patients with five or fewer prenatal visits was about double for patients with drug use between 1983 and 1986, and this gap widened from 1987 to 1990. By 1990, 45.6% of patients with drug use had five or fewer prenatal visits versus 17.2% of those without drug use. These differences were statistically significant for each year of analysis and for the total data set, whereas compared groups were matched for eight previously mentioned variables.

Preventable perinatal mortality (expressed as a percentage of perinatal deaths) rose over the 8-year period (Fig. 7). The largest increase was seen in black patients. By 1990, 42% of perinatal deaths were preventable (44% of black perinatal deaths versus 21% of white perinatal deaths). In Fig. 8 is the relationship shown between drug use and preventable perinatal mortality. Throughout the years of study patients with drug use had a two to three times higher incidence of prevent-

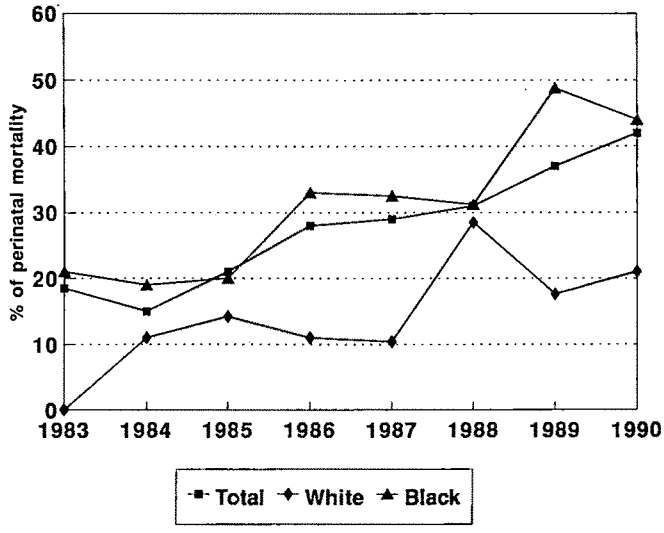


Fig. 7. Preventable perinatal mortality, 1983 to 1990.

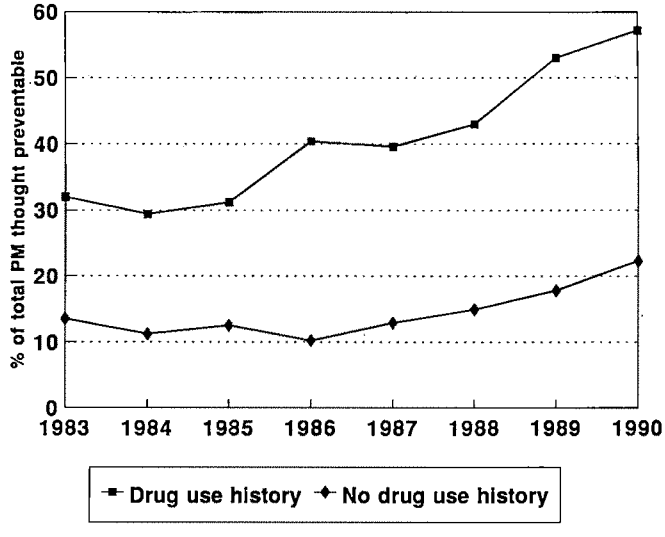


Fig. 8. Drug use and preventable perinatal mortality, 1983 to 1990.

able perinatal deaths than those without a history of drug use. The percentage of preventable perinatal deaths rose to 58.2% for drug users in 1990 versus 21.9% for patients without drug use. These differences were statistically significant for each year of analysis and the total data set, whereas compared groups were matched for eight previously mentioned variables.

The next analyses (Figs. 9, 10, and 11) separate the patients into three groups: group 1, drug use and five or fewer prenatal visits; group 2, drug use and more than five prenatal visits, group 3, no drug use and more than five prenatal visits. These groups were analyzed for their low-birth-weight rate, perinatal mortality, and preventable perinatal mortality.

The low-birth-weight rate (Fig. 9) in each year of analysis was the highest for drug users with five or fewer prenatal visits. In each year of analysis the incidence was about two to three times higher for group 1 (drug use, inadequate prenatal care) than for group 2 (drug use, adequate prenatal care). These differences were statistically significant for each year of analysis and for the total data set. No statistically significant differences were found between group 2 (drug use, adequate prenatal care) and group 3 (no drug use, adequate prenatal care), although low-birth-weight rates were 1% to 2% higher in the group with drug use. Obviously the most significant differences were seen between group 1 (drug use, inadequate prenatal care) and group 3 (no drug use, adequate prenatal care), as seen in 1990 with a 25.1% rate for group 1 versus 9.1% for group

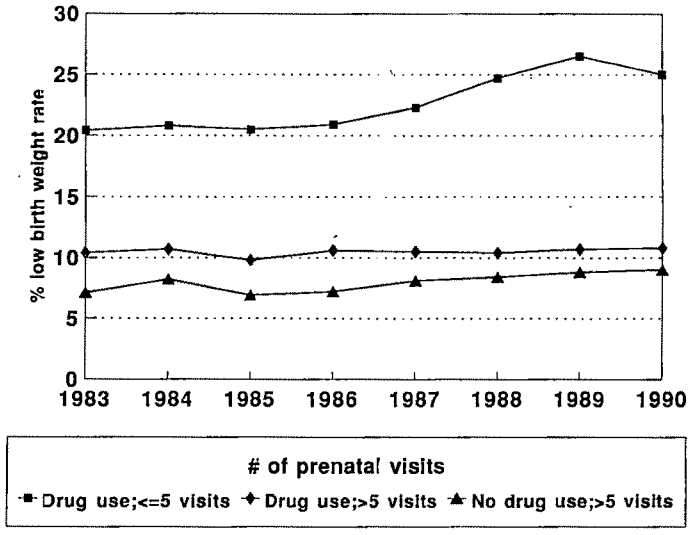


Fig. 9. Low birth weight, drug use, and prenatal care.

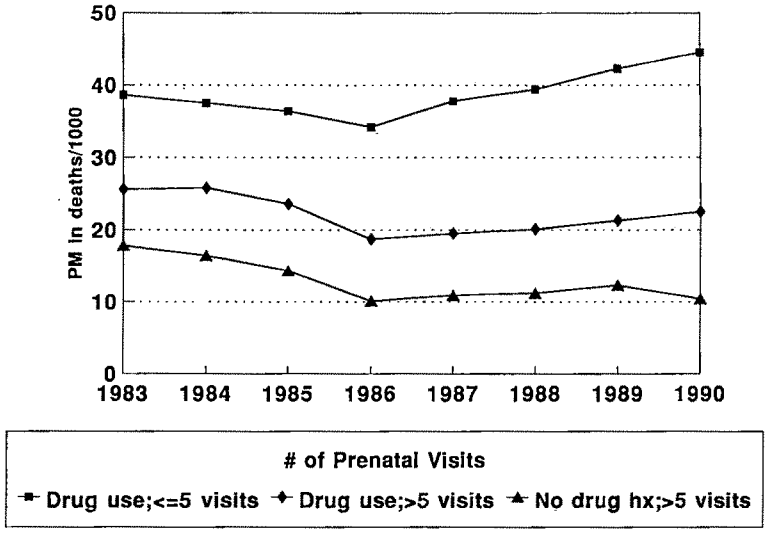


Fig. 10. Perinatal mortality, drug use, and prenatal care.

3. In all these comparisons groups were matched for the eight selected variables.

In a similar stratification the relationship between perinatal mortality, drug use, and number of prenatal visits is shown in Fig. 10. The perinatal mortality rate actually declined over the 8-year study period for patients with no drug use and more than five prenatal visits (from 17.9/1000 in 1985 to 10.6/1000 in 1990). Patients with drug use and more than five prenatal visits showed an initial decline from 1983 to 1986 and a slight increase from 1987 to 1990. Patients with a drug history and fewer than five visits had a two to four times higher incidence of perinatal mortality than patients without drug use and more than five prenatal visits. The differences between all groups were statistically significant, but the most pronounced differences were between group 1 with inadequate prenatal care and groups 2 and 3 with adequate prenatal care. Drug use had less effect (group 2 vs group 3) than adequacy of care (group 1 vs group 2). For example, in 1990 the perinatal mortality was 45.2 per 1000 for group 1, 22.4 per 1000 for group 2, and 10.6 per 1000 for group 3. In all these comparisons groups were matched for the eight selected variables.

Finally, in Fig. 11 the relationship is shown between preventable perinatal mortality, drug use, and number of prenatal visits. Throughout the years patients with drug use had a two to three times higher incidence of

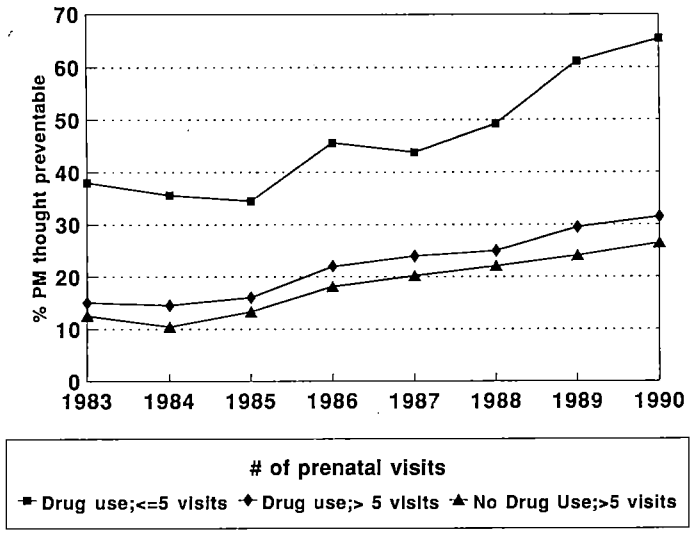


Fig. 11. Preventable perinatal mortality, drug use, and prenatal care.

preventable perinatal deaths if they had five or fewer prenatal visits when compared with patients with drug use and more than five prenatal visits. These differences were statically significant for each year and for the total data set. No significant differences were seen between groups 2 and 3 in each year and in the total data set. Significant differences were found in each year and in the total data set between group 1 versus group 2 or 3. In all comparisons groups were matched for eight selected variables. For example, in 1990 the percentage of preventable perinatal mortality was 65.4% for group 1, 31.2% for group 2, and 28.9% for group 3.

Comment

We conclude from these data that perinatal statistics worsened over the second half of the 1980s in one institution and urban community with persistent racial discrepancies in adverse outcome parameters. Crack cocaine arrived in Milwaukee in 1986. Illicit drug use and inadequacy of prenatal care increased significantly between 1986 and 1990, and a relationship between illicit drug use, inadequacy of prenatal care, and adverse perinatal outcome seems obvious. Illicit drug use during pregnancy increased, especially among women aged 18 to 35. This age group experiences inadequacy of prenatal care, perinatal mortality, and preventable perinatal mortality, as well as the most significant increases in low birth weight.

Our study made an effort to separate the perinatal outcome effects of inadequate prenatal care (arbitrarily defined as five or fewer visits) from the illicit drug use. Whereas drug use by itself had an adverse effect on low birth weight and perinatal mortality, the number of prenatal visits had a much greater influence on adverse outcome. Pregnant drug users with five or fewer prenatal visits had a two to four times higher incidence of preventable perinatal deaths, low birth weight, and perinatal mortality as compared with pregnant drug users with more than five prenatal visits.

Illicit drug use and addiction to alcohol in women have been strongly associated with low self-esteem, domestic abuse, a history of sexual abuse, and a chaotic life-style.[11, 12] We hypothesize that the number of prenatal visits for pregnant women with drug use is a marker of the degree of life-style chaos they experience and likely a marker of the severity of their drug use. As long as the patient is capable of obtaining adequate prenatal care while using drugs, immediate perinatal outcome is adversely affected on a limited scale. The patient is still reasonably "organizing" her life. The key ingredients are the organization and support systems that lead to an "adequate" number of prenatal visits. On the other hand, when prenatal care becomes clearly inadequate, extremely adverse effects are seen on immediate perinatal outcome. An important effect of illicit drug use in pregnancy is its influence on social behavior, incidence of sexually transmitted disease, inadequate nutrition, and compliance with prenatal care. In an urban setting existing socioeconomic circumstances often already place pregnancies at a higher risk for adverse outcome. While adverse socioeconomic circumstances cannot be resolved by medical interventions and root causes of illicit drug use among pregnant women (still rising in 1991 in many urban areas) are not affected by medical interventions, our data support the concept that outreach programs to actively recruit pregnant women with illicit drug use into prenatal care could obviate adverse pregnancy outcomes. These programs ought to be street corner and community based

and primarily address coping and chaos avoidance needs. The social chaos needs treatment first. The stage could then be set for adequate prenatal care and treatment of the addiction itself.

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Discussion

DR. WASHINGTON C. HILL, Nashville, Tennessee. The effects of substance abuse or chemical dependency on the pregnant woman and her fetus have been reviewed in several publications.[1,2] We are about to lose, if we have not done so already, many of the gains we have made because of drug use during pregnancy. Maternal risks, depending on the substance, can result in death, stroke, sexually transmitted diseases, human immunodeficiency virus infection, and other serious maternal diseases, to mention only a few. The risks to the fetus or neonate as mentioned by Dr. Broekhuizen include spontaneous abortion, renal anomalies and other congenital anomalies, preterm birth, low birth weight, perinatal death, sudden infant death syndrome, and full neonatal intensive care nurseries among other problems. We have learned recently that the sperm may even act as a vector to transport cocaine into an ovum.[3] The question these authors tried to answer is, What is the underlying reason for adverse pregnancy outcome in patients who abuse substances? Why do these problems occur? Is it the drug use, inadequate prenatal care, or something else? Dr. Broekhuizen suggests that the patient's use of drugs, be they illicit (a term that I do not like) or nonillicit, causes a chaotic life-style that precludes the patient obtaining adequate prenatal care. He and his colleagues feel therefore that adequacy of prenatal care is a marker of "social chaos" and affects outcome *more than* the drug use itself. I am not sure that this is really the case. The social chaos (a term that I do like) is created by drug use and a number of other factors in these patients' lives. Social chaos is a marker for other ills in the patient's life and environment. Why patients do not come in for prenatal care is complex, whether they do or do not abuse substances.[1,2] Why do some patients seek care and others not? We have known for years that the lack of adequate prenatal care, whatever that is, correlates with poor or adverse perinatal outcome. This is not new. Intensive prenatal care improves perinatal outcome in the chemically dependent woman. Whether a patient who is abusing substances comes in for prenatal care is affected equally by her support system, how much in control of her life she really is, the environment where the prenatal care is given, and other factors. A patient, even though she may be abusing substances, who has a good support system at home, who is in some control, and who is made to feel accepted at her prenatal care environment (our responsibility) will obtain and continue to seek prenatal care. The patient who has little or no support system, who is in poor control of her life, and whose prenatal care environment is not receptive to taking care of her either will not obtain prenatal care or will leave it. By the way putting the substance-abusing pregnant woman in jail, as has been proposed by some and abhorred by others, is not considered being receptive.

I believe it is not simply the number of prenatal visits, whether four, five, six, seven, or eight, that determines the perinatal outcome but these other factors as well. The number of prenatal visits certainly influences adverse outcome, but what we really need to get at is what influences the number of prenatal visits and what we need to do to increase that number. Dr. Broekhuizen is correct in his diagnosis, but we need to go further if we are to treat the problem effectively. Culturally sensitive outreach programs that actively recruit pregnant women into prenatal care will definitely obviate adverse pregnancy outcome. This article suggests, although slightly, that this is all we need to do. It really isn't! What is sorely needed are more adequate comprehensive prenatal treatment programs for these patients.[2] Clearly, there are all too few treatment centers and only a rare residential program for the drug-dependent pregnant woman. A successful approach to combat this problem must include unique prevention, treatment, and research efforts. The authors have attempted to tackle an elusive problem that is facing all of us who practice. Although there may be problems with the design of their study, they have given us food for thought, on which we need to go further.

I have the following questions for Dr. Broekhuizen:

1. Why use the term *illicit drug use?* Use of this term suggests that drugs which are not illegal are okay

to use during pregnancy—a point, however, I know Dr. Broekhuizen is not making.
2. What is the desired number of prenatal visits for optimal perinatal outcome? How did you come up with five?
3. Are there other reasons for the worsening perinatal statistics between 1983 and 1990?
4. What are the reasons and the possible cures for the disappointing persistent racial discrepancies?
5. What data support stating that crack cocaine arrived in Milwaukee in 1986 and illicict drug use increased significantly between 1986 and 1990? There is a National Institute on Drug Abuse report of a decline in the use of illicit drugs by women of childbearing age.[4]
6. There is a definite relationship between drug use, inadequate prenatal care, and adverse perinatal outcome. Where do resources, the degree of social support, and other factors fit into this formula?
7. Why did you exclude those who used alcohol and did you really do so?
8. Finally, and I know you have a quick answer for this one, what is "adequate prenatal care"?

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Dr. Sidney F. Bottoms, Detroit, Michigan. Dr. Broekhuizen and his colleagues have presented a very provocative article describing the interactions of drug abuse, inadequate prenatal care, and adverse perinatal outcomes. On the basis of their analysis, they conclude that adequacy of prenatal care is a marker of "social chaos" and that this affects outcome more than drug use itself. Although this conclusion may be correct, I have difficulty in accepting it on the basis of the data and analysis presented thus far. In my discussion I'll first cover a few technical considerations. After that, I'll address some practical issues and then present the authors with specific questions.

It seems that physicians often have the same warm feeling about statisticians that they do about attorneys. Sometimes, however, it is important to overcome our anxieties and seek the services of a professional statistician. I would like to cite a few of the reasons why I would recommend that the authors obtain a statistical consultation. Although the first sentence of the abstract indicates that trends in drug use were analyzed, all statistical comparisons were made between groups within a given year. An analysis of trends, of course, requires statistical comparison between years. There is also no information about how large the denominaotrs are for calculating rates. This leaves both the statistical reviewer and the reader at a loss to determine the degree to which these rates are subject to variation. For example, I believe that the authors would agree that a rate of 20% based on one out of five patients would be interpreted quite differently than a rate of 20% based on 100 out of 500 patients. Typically, large epidemiologic studies either have used a minimum size for denominators to calculate rates, have reported the actual size of the groups, or have presented confidence intervals to facilitate accurate interpretation of the data. There is also a multiple comparison problem; there are at lease 88 independent comparisons at $p < 0.05$. You would expect that 5% of the 88 comparisons or a total of four "significant" results would actually be due to chance alone (statistical "flukes"). A more sophisticated statistical model, a hierarchical design, or a lower p value considered significant is frequently used by statisticians to minimize this type of problem.

There are also several practical issues in interpreting the data that must be considered. The authors have compared perinatal mortality rates in a control population with adequate prenatal care, drug abusers with adequate prenatal care, and drug abusers without adequate prenatal care. The last of these groups had a higher perinatal mortality rate. They interpret this to mean that the adequacy of prenatal care is a marker for "social chaos" and that it affects outcome more than drug use itself. The authors failed to provide any information about drug-free patients with inadequate prenatal care. Without this comparison group, it is impossible to determine whether the authors' conclusion is warranted. Often, this drug-free group without adequate prenatal care has many pregnant adolescents who, in spite of their lack of prenatal care, seem to fair better than drug abusers. To draw their conclusion, the authors have implied that drug abusers who receive adequate prenatal care are otherwise comparable to drug abusers with inadequate care. Typically, this is not the case. Substance abusers who receive adequate prenatal care are likely to be lighter users, to marijuana rather than crack cocaine or heroin, and to have decreased drug use during pregnancy. Although I could cite references for this point, I doubt that anyone would argue that a woman "high" on cocaine or heroin is less likely to make or keep an appointment for prenatal care than one in complete command of her senses.

Finally, I have three specific questions for the authors. First, do the authors have any data reporting outcome according to the specific type of drug abuse? Information on this subject would be helpful in determining whether drug abusers with and without prenatal care were comparable. The second question is in regard to whether the authors have made any comparisons controlling for such important factors as socioeconomic status, race, alcohol abuse, or smoking. Known associations of substance abuse and inadequate prenatal care with one or more of these factors could account for the effects ascribed by the authors to "social chaos." Finally, do the authors have any information regarding perinatal outcome among drug-free women with inadequate prenatal care?

Dr. Pedro A. Poma, Melrose Park, Illinois. We know that for every patient we find who has a history of drug

use there are >10 women who may be found by routine screening. At what prevalence level in the community do you recommend we must do routine screening?

PRESIDENT YOUNGBLOOD. I would just add my own comment. In 1983, our low-birth-weight rate was about 15%. We watched it go down until about 1986 or 1987 to 11%, and it has been on the rise since to 15%.

DR. BROEKHUIZEN (Closing). I thank both Dr. Hill and Dr. Bottoms for their thoughtful comments and criticisms; I knew that there were some valid criticisms of this study.

Dr. Hill suggested that our conclusion was that simply the number of prenatal visits affected outcome. If that was the conclusion, I probably ought to rewrite the Comment section of the article, because that would be an unfortunate conclusion.

In fact, I feel that our data support Dr. Hill's belief that the social support system and the support within the prenatal care environment are the first two ingredients for prenatal care for a disadvantaged population. I thought we concluded the same.

The perinatal statistics for patients without drug use but with inadequate prenatal care, and I can answer Dr. Bottoms' third question right here, are still unacceptably high in our institution. If we had included them in the statistics, and I accept the criticism that we should have, they were better than in the group with drug use and inadequate prenatal care but worse than in the group with drug use and adequate prenatal care.

Our point is that drug use is associated with even more social chaos than in those patients with just inadequate prenatal care, and the social chaos needs to be addressed.

Another of Dr. Hill's questions pertained to illicit drug use as a term. I do not like that term either. We were asked to do the study to come up with some rationale for funding, and we used this data base for birth certificate information. The subcategory that is used for the birth certificate in Wisconsin is "illicit" drug use and we were tied to that particular category.

Five prenatal visits as a cutoff and what is adequate prenatal care—this was done initially because the average number of prenatal visits in all the years was 9.9, so we thought we would take half. When we analyzed differences between groups at one, two, three, four, and five prenatal visits, we started to lose any statistical significance at about five. So five was the highest number at which we could find statistical differences.

Are there other reasons for worsening perinatal statistics? Increasing unemployment, crime, poor housing, less-accessible Medicaid programs since 1983 in our community, health maintenance organization–run Medicaid programs, and in the last year a provider shortage have contributed to these statistics.

Reasons and cures for persistent racial discrepancies—another one of Dr. Hill's questions—socioeconomic differences, still very much along racial and economic lines. This applies as well to access to medical care. These are the root problems and they are compounded by lack of cultural sensitivity in many medical care settings. That's my diagnosis. I think we all have to come up with the cure.

Data to support that crack cocaine hit Milwaukee in 1986? Although our police department's performance is somewhat in question lately, criminal statistics, halfway houses, and drug rehabilitation settings provided that information.

Alcohol, did we really include or exclude? We actually used alcohol and tobacco use as control variables. The same is true for race and age. So, and this might answer Dr. Bottoms' second question to some extent, we matched the patients for age, race, and about eight other variables in all comparisons. We might want to make that a little bit more clear in the final version of the article.

What is adequate prenatal care? Dr. Hill asked for a quick answer. I'll try. A minimum of pure medical content can be defined, but I think that in most cases it will have to be individualized and dependent on the health, knowledge, and life-style of individual patients.

I do know that patients with adverse pregnancy outcome in my institution need a lot more care than just medically defined care, which would be sufficient for my suburban neighbor, and that's about the best way I can answer that question.

Dr. Bottoms, I have to accept having been a somewhat poor student of some of your statistical courses. I accept your criticism regarding the reporting of the statistics. Now, with several hardware and software transitions that we experienced in our institution over the last 8 years and actually a move of the whole institution, it was impossible to do multivariate analysis. I accept some weaknesses in our statistical approach. Nevertheless, I believe that they do not interfere with the validity of our conclusions.

Dr. Poma, at what prevalence rate in the community do I recommend routine screening? I don't have an answer to that. I really think you have to define that in your own community.

And last, in answer to Dr. Youngblood's comment, I think one of the more tragic things that permeates this study may not be the question of drug use versus inadequate prenatal care. The more tragic feature may be the persistence of racial discrepancies and the worsening of perinatal statistics over the last 4 or 5 years in our urban environment.

Eclampsia

VII. Pregnancy outcome after eclampsia and long-term prognosis

Baha M. Sibai, MD, Cem Sarinoglu, MD, and Brian M. Mercer, MD
Memphis, Tennessee

OBJECTIVES: Our goal was to report pregnancy outcome and long-term prognosis after eclampsia.
STUDY DESIGN: Women whose pregnancies were managed at the E.H. Crump Women's Hospital between August 1977 and April 1989 were studied. A total of 223 women with eclampsia underwent follow-up for an average of 7.2 years. Thirteen had preexisting hypertension and 210 were normotensive (31 were multiparous and 179 were nulliparous).
RESULTS: Among these women 23 who were multiparous and 159 who were nulliparous had 366 subsequent pregnancies: 22% of pregnancies were complicated by preeclampsia, 1.9% by eclampsia, and 2.5% by abruptio placentae; 2.7% resulted in perinatal death. Within the nulliparous group, women who had eclampsia before 37 weeks' gestation in the index pregnancy had significantly higher incidences of preeclampsia and poor perinatal outcome in subsequent pregnancies as compared with those who had eclampsia at ≥37 weeks' gestation; the highest incidence of obstetric complications occurred in those having eclampsia at ≤30 weeks. Twenty of the 210 normotensive women (9.5%) had chronic hypertension on follow-up; the highest incidence (17.9%) being in those with eclampsia at ≤30 weeks and the lowest incidence (4.8%) in those having eclampsia at ≥37 weeks. Women with eclampsia who had preeclampsia in subsequent pregnancies had a higher incidence of chronic hypertension as compared with those who were normotensive in subsequent pregnancies (25% vs 2%, $p < 0.0001$). Long-term maternal complications included dialysis required in one patient and one case of cardiomyopathy in women with chronic hypertension; there was one maternal death in a woman with chronic hypertension. None of the women had evidence of neurologic deficit or seizures during follow-up.
CONCLUSIONS: These findings should be used in counseling women who have had eclampsia and are considering future pregnancies. (Am J Obstet Gynecol 1992;166:1757-63.)

Key words: Eclampsia, recurrence, remote prognosis

Eclampsia is becoming a rare complication of pregnancy in the Western world.[1,2] However, it continues to be a major health problem in developing countries,[3,4] as well as among young indigent patients in the United States.[5,6] Pregnancies complicated by eclampsia are still associated with increased perinatal mortality and morbidity.[7] In addition, such pregnancies are usually also associated with increased maternal morbidity: hemolysis, pulmonary edema, disseminated intravascular coagulopathy, cerebral injury, and acute renal failure.[3,7]

The development of eclampsia, particularly when associated with poor maternal-perinatal outcome, is a frightening experience for both the patient and her family members. As a result, the managing physician must be prepared to answer questions regarding outcome in future pregnancies, as well as any potential long-term residual damage from eclampsia and/or its complications. The purpose of this study is to report subsequent pregnancy outcome and remote maternal prognosis of 223 eclamptic women.

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6/6/37126

Material and methods

E.H. Crump Women's Hospital and Perinatal Center in Memphis serves as a tertiary referral center for an area that includes five states: Tennessee, Arkansas, Mississippi, Missouri, and Kentucky. During the time period from August 1977 to April 1989, 254 of the pregnancies managed at this center were complicated by eclampsia. The diagnosis, management, and maternal-perinatal outcome of these patients were previously described.[5] One patient died during the acute eclamptic episode, and one patient died of massive pulmonary embolism at about 9 weeks post partum after admission to a local hospital. This latter patient had tricuspid atresia with secondary pulmonary hypertension.

Twenty-nine of the 252 surviving patients either had inadequate medical follow-up data or were lost to fol-

Table I. Pregnancies after eclampsia in women without preexisting chronic hypertension

Outcome	*Nulliparous women (n = 159)‡*		*Multiparous women (n = 23)*		*Total (N = 182)*	
	No.	*%*	*No.*	*%*	*No.*	*(%)*
No. of pregnancies	334		32		366*	
Normotensive pregnancies	254	76	25	78	279	76.2
Preeclampsia-eclampsia	80	24	7	22	87	23.8
Mild	43	12.9	5	15.6	48	13.1
Severe	30	9.0	2	6.3	32	8.8
Eclampsia	7	2.1	0	0	7	1.9
Preterm (<37 wk)	48	14.4	2	6.3	50	13.7
IUGR (<10th percentile)	20	5.9	2	6.3	22	6.0
Abruptio placentae	9	2.7	0	0	9	2.5
Perinatal deaths	10	3.0	0	0	10	2.7

IUGR, Intrauterine growth retardation.
*There were 370 births (four sets of twins), all in nulliparous women.

low-up. The remaining 223 patients had adequate medical follow-up data for at least 2 years after eclampsia. These latter patients served as the study population for this report.

Early follow-up (first 6 weeks) after delivery was done at our obstetric clinics, whereas subsequent follow-up data were obtained from our clinics, local physicians, local health departments, and local hospital records. In the majority of cases the patients were seen and followed up by B.M.S. Maternal evaluation included an interval history with special emphasis on history of convulsions and/or neurologic deficit, use of anticonvulsive and/or antihypertensive medications, and future pregnancy outcome. Outcome in subsequent pregnancies was reviewed with regard to incidences of preeclampsia-eclampsia, abruptio placentae, and perinatal outcome. Only pregnancies delivered beyond the first trimester were included in the analysis.

All data pertaining to blood pressure measurements during subsequent physical examinations and/or pregnancy were obtained. The diagnosis of chronic hypertension was based on the presence of documented hypertension (either before the index pregnancy or on follow-up) for which the patient received antihypertensive therapy. This diagnosis was usually made when the patient had persistent elevations of diastolic pressure of at least 90 mm Hg on two occasions at least 6 months apart.

Data analysis was performed with X^2 or Fisher's exact test and odds ratios. A p value of <0.05 was considered significant.

Results

Thirteen women had preexisting chronic hypertension and/or renal disease before the onset of eclampsia. Eleven were black and two were white. Seven were multiparous and six were nulliparous. The mean age of onset of eclampsia in these patients was 25.2 years (range 14 to 36 years), and the mean duration of follow-up was 7.2 years (range 3 to 13 years).

Six of the 13 women had no subsequent pregnancies, and the other 7 had a total of 13 subsequent pregnancies. Of these 13 pregnancies 7 (53.8%) were complicated by severe preeclampsia, 5 (38.5%) were complicated by mild hypertension, and one was normotensive. Four (30.8%) of the 13 resulted in perinatal death, four were complicated by fetal growth retardation (<10th percentile for gestational age), and one (7.7%) was complicated by abruptio placentae.

The mean maternal age at time of last follow-up in these 13 women was 32.4 years (range 19 to 45 years). In one patient hypertensive cardiomyopathy developed after 3 years of follow-up, and progressive renal failure necessitating dialysis developed 6 months after eclampsia in one patient with biopsy-proved glomerulosclerosis. This patient is still undergoing dialysis after 5 years of follow-up. One additional patient died of cardiovascular complications after 13 years of follow-up. In addition, none of the surviving women had evidence of neurologic deficit or seizures on follow-up.

A total of 210 women were normotensive at the time of onset of eclampsia. Forty-one (19.5%) were white, 168 (80%) were black, and one was Asian. Thirty-one (14.8%) were para ≥1 and 179 (85.2%) were nulliparous. The mean age at onset of eclampsia in these patients was 18.0 years (range 11 to 34 years), and the mean duration of follow-up was 7.2 years (range 2 to 13 years).

Twenty nulliparous women had no subsequent pregnancies, and the other 159 had a total of 334 subsequent pregnancies. Eight multiparous women had no future pregnancies, and the other 23 had a total of 32 subsequent pregnancies. Table I summarizes the pregnancy outcome in these women according to parity. The overall incidence of preeclampsia-eclampsia in subsequent pregnancies was 23.8%. There was no difference

Table II. Outcome in subsequent pregnancies in nulliparous women according to gestational age at onset of eclampsia in index pregnancy

	Weeks' gestation							
	≤30		*31-36*		*37-41*			
	No.	*%*	*No.*	*%*	*No.*	*%*	*Significance**	*Odds ratio**
No. of women	26		55		78			
No. of pregnancies	54†		116†		164‡			
Preeclampsia-eclampsia	24	44.4	39	33.6	17	10.4	<0.0001	6.9:1
Mild	9	16.7	25	21.6	9	5.5	<0.01	3.4:1
Severe	14	25	12	10.3	4	2.5	<0.0001	14:1
Eclampsia	1	1.8	2	1.7	4	2.4	NS	—
Preterm (<37 wk)	19	35.2	18	15.5	11	6.7	<0.0001	7.5:1
IUGR	10	18.5	6	5.2	4	2.5	<0.0001	8.4:1
Abruptio placentae	4	7.4	4	3.4	1	0.6	<0.004	13:1
Perinatal deaths	3	5.5	4	3.4	3	1.8	0.15	3.1:1

IUGR, Intrauterine growth retardation (<10th percentile).
*Comparison of ≤30 versus 37 to 41 weeks' gestation.
†One set of twins in each group.
‡Two sets of twins (166 births).

in this incidence between women who had eclampsia as nullipara and those who were multipara; however, all cases of repeat eclampsia occurred in the nulliparous group. In addition, all cases of abruptio placentae and perinatal deaths occurred in the nulliparous group.

Table II compares outcome in subsequent pregnancies in nulliparous women according to gestational age at the time of onset of eclampsia in the index pregnancy. The women who had eclampsia before 37 weeks' gestation had significantly higher incidences of both mild and severe preeclampsia in subsequent pregnancies as compared with women who had eclampsia at ≥37 weeks' gestation. In addition, this group of women had a significantly higher incidence of poor perinatal outcome in subsequent pregnancies. Moreover, women who had eclampsia at ≤30 weeks' gestation had the highest incidence of preeclampsia and the worst perinatal outcome in subsequent pregnancies.

The mean maternal age at the time of last follow-up in the 210 women who were normotensive at the time of eclampsia was 25.2 years (range 17 to 43 years). In 3 of the 31 multiparous women (9.7%) and 17 of the 179 nulliparous women (9.5%) chronic hypertension developed during follow-up. The overall incidence of chronic hypertension was 9.5%.

For the 179 nulliparous women, the data were subsequently analyzed according to gestational age at time of onset of eclampsia in the index pregnancy. Twenty-eight of these women had eclampsia at ≤30 weeks, 68 at 31 to 36 weeks, and 83 at ≥37 weeks. The average durations of follow-up among these three groups were 7.8, 7.0, and 7.3 years, respectively. Fig. 1 describes the incidence of chronic hypertension among the various groups. Within this group, women who had eclampsia at ≤30 weeks' gestation had a significantly higher incidence of chronic hypertension as compared with those who had eclampsia at ≥37 weeks ($p < 0.04$, odds ratio 4.3:1). In addition, women who had eclampsia at 31 to 36 weeks' gestation had a higher incidence of chronic hypertension as compared with those who had eclampsia at ≥37 weeks. However, this difference did not reach statistical significance ($p = 0.11$, odds ratio 2.6:1).

For the nulliparous women who had subsequent pregnancies ($n = 159$), the data regarding development of chronic hypertension were analyzed according to outcome in subsequent pregnancies. In 16 (10%) of these women chronic hypertension developed on follow-up. Within this group of women, 103 had normotensive subsequent pregnancies and 56 had at least one subsequent pregnancy that was complicated by preeclampsia. In 2 of the 103 women with normotensive subsequent pregnancies (2%), hypertension developed, whereas hypertension developed in 14 (25%) of the 56 women with subsequent preeclampsia ($p < 0.0001$, odds ratio: 22.1).

There were no maternal complications on follow-up in these patients, and none of the women had evidence of neurologic deficit or seizures on follow-up.

Comment

The findings of this study indicate that women in whom eclampsia develops are at increased risk of preeclampsia-eclampsia and abruptio placentae in subsequent pregnancies. The risks were particularly increased in those women who had preexisting chronic hypertension before the onset of eclampsia. In these women the risk of severe preeclampsia in subsequent

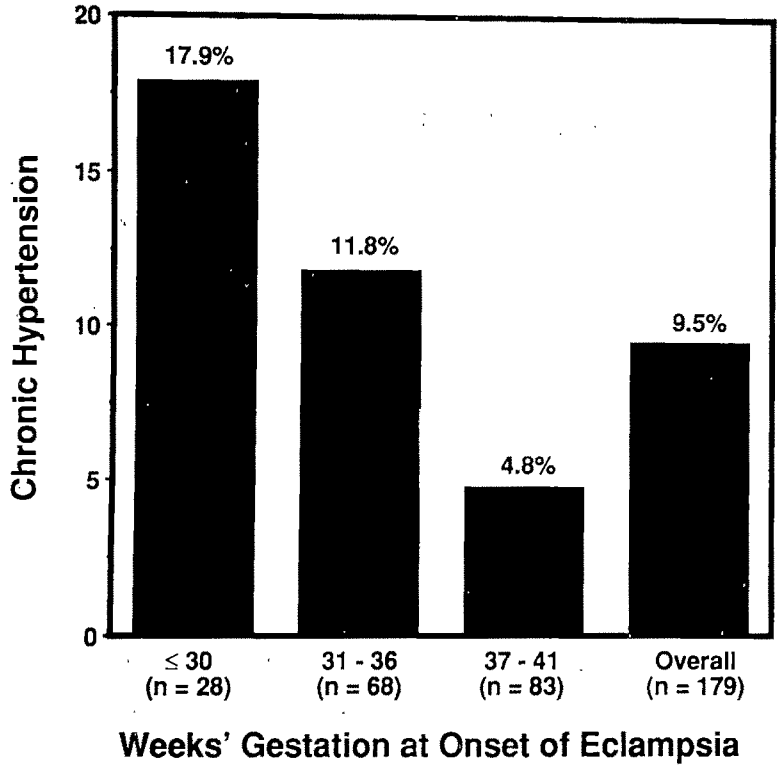


Fig. 1. Incidence of chronic hypertension on follow-up in nulliparous women according to gestational age at time of onset of eclampsia.

Table III. Pregnancy after eclampsia

	Chesley[9]	*Lopez-Llera and Horta*[10]*	*Adelusi and Ojengbeda*[11]*	*Current study*
No. of women	171	110	64	182
No. of pregnancies	398	110	64	366
Preeclampsia (%)	23.1	35.4	26.6	21.9
Eclampsia (%)	1.0	—†	15.6	1.9
Abruptio placentae (%)	2.0	—†	—†	2.5
Preterm delivery (%)	6.5	—†	34	13.7
IUGR (%)	—†	22	12.5	6.0
Perinatal deaths (%)	4.7	3.6	6.25	2.7

*Follow-up in only one subsequent pregnancy.
†Data not available.

pregnancies was 53.8%, whereas the risk of abruptio placentae was 7.7%. In addition, these women had an increased rate of perinatal death (30.8%) and an increased incidence of fetal growth retardation (30.8%) in subsequent pregnancies. Thus they should be informed of the high likelihood of severe preeclampsia and poor perinatal outcome in subsequent pregnancies. Moreover, such women should be informed of the increased long-term maternal morbidity and counseled regarding close medical follow-up.

For the women without chronic hypertension before the onset of eclampsia, the risk of preeclampsia in subsequent pregnancies was 21.9%, the risk of eclampsia was 1.9%, and the risk of abruptio placentae was 2.5%. The risks were similar whether the patient had eclampsia when nulliparous or multiparous. However, the incidence of these obstetric complications was more than threefold higher than the expected incidence for similar complications in the general obstetric population at this hospital. In addition, within the group who had eclampsia when nulliparous, the risk of obstetric complications in subsequent pregnancies was related to the time of onset of eclampsia (Table II). Women who had eclampsia at ≤30 weeks' gestation had increased rates of severe preeclampsia, abruptio placentae, preterm birth, and perinatal death in their subsequent pregnancies, whereas women who had eclampsia at ≥37 weeks' gestation had only an increased rate of eclampsia

(2.4%) in their subsequent pregnancies. These findings support our previous observations in a similar study[8] and should be used in counseling those women who are considering future pregnancies.

During the past 20 years there have been three studies describing pregnancy outcomes after eclampsia.[9-11] The findings of these studies, as well as those of the current study, are summarized in Table III. It is important to note that the findings of this study are almost identical to those reported by Chesley.[9] The other two studies reported from Mexico and Nigeria found higher incidences of preeclampsia-eclampsia, preterm birth, and fetal growth retardation as compared with our findings. These differences may be related to differences in populations studied, as well as to management used.

The long-term effects of eclampsia on maternal blood pressure have been extensively studied by Chesley,[9] who concluded that eclampsia does not lead to chronic hypertension later in life. The results of this study show that women without evidence of chronic hypertension before eclampsia have a 9.5% incidence of chronic hypertension on follow-up. The incidence was similar whether the patient had eclampsia when nulliparous or when multiparous. In addition, this incidence was similar to the respective incidence in the general female population (age groups 25 to 34 years).[8] On the other hand, the incidence of chronic hypertension was significantly higher in those who had eclampsia at ≤30 weeks' gestation (17.9%) than in those who had eclampsia at ≥37 weeks' gestation (4.8%). Moreover, in women who had subsequent pregnancies, the incidence of chronic hypertension was significantly higher in those who had subsequent pregnancies complicated by preeclampsia (25%) than in those who had normotensive subsequent pregnancies (2%). These findings may be useful regarding the counseling of these women about future medical follow-up.

None of the surviving women had evidence of neurologic deficit or seizures on follow-up from 2 to 13 years. These findings are in disagreement with the conclusions of Sexton,[12] who suggested an association between eclampsia and future development of epilepsy. This difference might reflect differences in short-term management of eclampsia and its complications.

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Discussion

DR. NORMAN F. GANT, Dallas, Texas. I enjoyed the well-prepared study by Sibai et al. This report continues the saga of the management of eclampsia by this group of investigators in Memphis and is an original study of the outcome in 223 patients with eclampsia. The outcome follow-up ranged from 2 to >13 years.

The study design included a separation of patients into those who had chronic hypertension at the time of eclamptic seizures; there were 13 such patients. There were 210 patients who had been normotensive before eclamptic seizure. The outcome, with respect to subsequent pregnancies, development of hypertension, neurologic complications, and other long-term consequences, was then assessed with respect to those patients who had chronic hypertension at the time of seizure and the outcomes that were observed in those women who had been normotensive before eclamptic seizure. Additionally, the patients were further divided into nulliparous patients and multiparous patients at the time of eclamptic seizure. Finally, those patients who were nulliparous at the time of seizure were then further divided into the time of onset of the eclamptic seizure (i.e., <30 weeks, between 30 and 37 weeks, and >37 weeks). Sibai et al. then compared their results with three previously published, similar studies of outcome in patients with eclampsia.

In summary, Dr. Sibai observed that those women who had chronic hypertension at the time of eclamptic seizure fared very poorly. There was one death in this group and there was a high incidence of repeat severe hypertension in subsequent pregnancies. There were, interestingly enough, no neurologic sequelae, but there was one renal failure and one severe cardiomyopathy.

With respect to those patients who had been normotensive before eclamptic seizure, the outcome, as previously mentioned, was separated into those women who were nulliparous and multiparous at the time.

There appeared to be no difference between nulliparous and multiparous women in long-term outcome variables, at least for the duration of the study. In those women who had been nulliparous at the time of seizure, there was a significantly higher incidence of later hypertension and recurrent hypertension in those women who had eclamptic seizure at ≤30 weeks, compared with those who had eclamptic seizure at 30 to 37 weeks and those who had seizure after 37 weeks. Finally, there was approximately a 2% recurrence of eclampsia in each of the three groups of women separated by gestational age at the time of previous eclamptic seizure.

The results of this interesting study compared most favorably with those obtained by Chesley in his now-famous long-term follow-ups cited by the authors in their report.

I have only two questions for the authors. First, in spite of the tremendously worse outcome variables in the patients with chronic hypertension at the time of eclamptic seizure, I noted that there were no repeat eclamptic seizures in this group of individuals. Was this because the numbers were too few, or was this because these patients underwent close follow-up in subsequent pregnancies? Finally, I am amazed at the number of cases of eclampsia that Sibai et al. continue to accrue in Memphis. I wonder if there has been a decrease in the incidence of eclampsia in their population since they instituted an increased dosage of magnesium sulfate to 2 gm/hr as prophylaxis against eclamptic seizures?

Dr. Jennifer R. Niebyl, Iowa City, Iowa. Dr. Sibai has taught us a great deal about eclampsia, and this is the seventh in a series of articles describing this disorder. He has previously reviewed the maternal and perinatal outcomes in the index pregnancies of 254 patients with eclampsia.[1] He now presents long-term follow-up of both maternal status and fetal outcome in subsequent pregnancies of 223 of these individuals. They have had follow-up for a mean of 7.2 years with a range of 2 to 13 years.

Of note is the 1.9% recurrence rate of eclampsia, in a total of seven patients. Sibai et al.[2] previously reported that 31% of cases of eclampsia are not preventable with standard prenatal care. My questions for Dr. Sibai are: Did the physicians make any adjustments in prenatal surveillance in subsequent pregnancies? Did these patients with previous eclampsia make more frequent visits in their subsequent pregnancies? Was there more aggressive use of magnesium sulfate therapy in the subsequent pregnancies or was there longer postpartum observation? What are your recommendations for prenatal care in women at increased risk for eclampsia?

Bryans et al.[3] carried out follow-up of women who had eclampsia and were treated at the Medical College of Georgia; they found no increase in subsequent chronic hypertension in these patients above that expected in the general population. Chesley et al.[4] monitored women with eclampsia at the Margaret Hague Hospital in New Jersey from 1931 to 1951, and they also concluded that eclampsia does not cause hypertension. This article reported that the risk of chronic hypertension is increased in this population, especially in those who had eclampsia before 30 weeks. Dr. Sibai, what is the risk of subsequent essential hypertension in your population without eclampsia? How great is the increased risk over the background risk? Why is this different from previous studies?

I look forward to an article reporting that resources are available and access to prenatal care is such that eclampsia has finally been eliminated in Tennessee and adjacent states.

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Dr. James N. Martin, Jr., Jackson, Mississippi. I have two questions for you regarding this excellent report. Do you think that the occurrence of preeclampsia earlier than 30 weeks in gestation is a marker of underlying chronic hypertensive disease in the first place? Is it not the horse rather than the cart?

Second, along the lines of Dr. Niebyl's comment and on the basis of your work with low-dose aspirin and calcium supplementation, do you think that modification of prenatal care for these special patients should include one or both of those substances or something else?

Dr. Steven E. Calvin, Minneapolis, Minnesota. I have a brief question. The assay methods for—and the understanding of—antiphospholipid antibodies have changed over the course of this study. Do you have any information about the presence of antiphospholipid antibodies, especially in the subset of <30 weeks?

Dr. Allan G. Charles, Chicago, Illinois. Considering the wide geographic area covered and the difficulties in follow-up of indigent patients, I wonder how Dr. Sibai has been able to monitor such a large number of patients for such a long period of time.

Dr. Sibai (Closing). I thank Dr. Gant and Dr. Niebyl for their kind remarks. Regarding the comments of Dr. Gant as to why we didn't see eclampsia in the 13 pregnancies in women who had previous chronic hypertension, there are probably two reasons.

First, the numbers are too small, only 13 pregnancies. Second, women who have chronic hypertension tend to have more close and intensive follow-up, and once (superimposed) preeclampsia develops, we become

very aggressive regarding indications to deliver them. This would probably explain why we didn't see it.

Regarding magnesium sulfate, I'm also surprised that we continue to see these cases of eclampsia because I would think the number would decrease. In Memphis, this hasn't changed in >40 years. We are still seeing the same problem. So the effort has to be directed to preventing preeclampsia, which is the first step before the onset of eclampsia. There is no doubt that after we increased magnesium sulfate to 2 gm/hr (described in my original report in 1981) the incidence of convulsions in women with preeclampsia who were receiving magnesium sulfate was about 1 in 125 women. Since 1985, when we started the 6 gm loading dose and 2 gm/hr, the incidence has fallen to about 1 in 400.

Dr. Niebyl brought up a very interesting point regarding recurrence. Is there any way we could have prevented recurrence in these seven nulliparous patients? Looking at the data in these patients, I guess better care could have prevented recurrence in about three of those seven.

For example, one of the patients had the first onset of eclampsia at 33 weeks. The second one had prenatal care for two visits. The third time she came, she had a blood pressure of 240/140 mm Hg and proteinuria (4+). The problem was she was only 18 weeks' gestation, and the physicians that consulted didn't really think of preeclampsia. So they treated the high blood pressure and did everything possible, thinking it would be renal disease. Magnesium sulfate was not used, and then eclampsia developed at that early stage. This patient had follow-up and she is still normotensive.

Few other patients have eclampsia develop as late-onset. They have preeclampsia. They receive magnesium sulfate, and then 3 or 4 days later eclampsia develops. It's very difficult to see how we could have prevented these cases, and we cannot blame the physicians who were taking care of them.

Interestingly, one other case was followed up at a local hospital. She had late-onset eclampsia the first time. The second time preeclampsia developed. She was given magnesium sulfate for 12 hours. Then magnesium sulfate was stopped, and eclampsia developed 19 hours later. This patient probably should have received magnesium sulfate ≥24 hours.

I think this corresponds with the comments of Dr. Martin. Maybe what we need to do more than anything is to prevent preeclampsia, because almost all of these women have preeclampsia. Currently, I don't know whether aspirin or calcium is going to prevent preeclampsia. There are trials going on at the present time, so we have to await the results.

To date the trials with calcium have not produced any benefit regarding preeclampsia. They have shown some benefit regarding the incidence of pregnancy-induced hypertension.

Dr. Niebyl mentioned the 9.5% incidence of chronic hypertension as being increased. I don't think this 9.5% incidence is slightly increased. If we look at the incidence using vital statistics for black patients in the United States, this incidence corresponds to that for the age group 25 to 34 years. The average age group in this follow-up was 25, so it seems our patients fit in the upper limit.

This agrees with the important point, which is that I don't believe eclampsia causes hypertension. I think what we are seeing is that eclampsia is like gestational diabetes. Women in whom severe preeclampsia or eclampsia develops early in gestation are at increased risk to have repeated episodes later on. Actually, these women might have had previously silent or underlying chronic hypertension or renal disease. In patients with either severe preeclampsia or eclampsia at <30 weeks, there is no doubt that a very large percentage of these women will have chronic hypertension or some form of renal disease. I don't think it has anything to do with eclampsia. It just became manifest during pregnancy.

There was a question about antiphospholipid antibodies. We have measured antiphospholipid antibodies in >200 women, particularly those who have the disease very early; I have yet to see a single positive test result. I personally do not think that there is a strong association the way it was previously stated in the literature. I think women who have antiphospholipid antibodies tend to have a syndrome similar to preeclampsia, but the reverse is not true.

Finally, in response to the question about close follow-up, you would be amazed, when you have a close relationship with these patients and you have been keeping in touch with them and their children, that they are very compliant with follow-up. Also, they were very good in bringing their children for follow-up, particularly those who were delivered of preterm babies. So, no, I'm not surprised that we were able to do that.

Preoperative and postoperative analysis of site-specific pelvic support defects in 81 women treated with sacrospinous ligament suspension and pelvic reconstruction

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Temple, Texas

OBJECTIVES: The objectives of this study were to identify factors that predict long-term success, defined as the absence of anatomic defects, in women undergoing pelvic reconstruction and to identify which defects most frequently persist or recur.

STUDY DESIGN: Eighty-one women treated by sacrospinous ligament suspension and pelvic reconstruction between 1984 and 1990 had site-specific analysis performed preoperatively and at consecutive postoperative visits. The findings at the 6-week postoperative visit and subsequent visits were compared for each of five sites: urethra, bladder, cuff, cul-de-sac, and rectum.

RESULTS: The Fisher exact test showed that patients with no support defects at the 6-week visit were less likely to require subsequent repair than patients who had any defect at that visit ($p = 0.003$). Thirteen patients with subsequent or persistent cystoceles had no bladder repair performed at the time of the study procedure. However, even with repair the bladder was the most likely site for persistence or recurrence.

CONCLUSION: Absence of any pelvic support defect at the 6-week postoperative visit is associated with a 3% likelihood that the patient will require subsequent reconstructive surgery within 2 to 5 years. The anterior segment provides the greatest challenge to restoration of normal anatomy. (AM J OBSTET GYNECOL 1992;166:1764-71.)

Key words: Pelvic support defects, sacrospinous ligament suspension, anatomic assessment, pelvic support index

Every honest surgeon of extensive and long experience will have to admit that he is not entirely and absolutely satisfied with his long term results of all his operations for prolapse and allied conditions.

Richard TeLinde[1]

Nichols[2] and Morley and DeLancey[3] have reported excellent success with sacrospinous ligament suspension of the prolapsed vaginal cuff. Morley and DeLancey observed that 22% of their patients monitored >1 year had "mild to moderate cystoceles" or "symptomatic cystoceles." Nichols has not specifically commented on failures other than "recurrent eversion."

Baden et al.[4] previously reported a pelvic profile based on analysis of six individual sites in the pelvis: urethra, bladder, cervix or cuff, cul-de-sac, rectum, and perineum. All sites are scored with the patient recumbent and straining down, allowing the surgeon to grade sites under stress. We have used a modification of their vaginal profile to assess five sites—urethra, bladder, cervix or cuff, cul-de-sac, and rectum—to analyze our patients with pelvic support defects. Nagata and Kato[5] used a similar scoring system to evaluate nine patients who had sacrospinous ligament suspension procedures.

The purpose of this project was to describe site-specific pelvic support defects in patients who have had sacrospinous ligament suspension of the vaginal cuff and pelvic reconstruction. Preoperative and postoperative assessments of each vaginal site were compared to evaluate factors that affect immediate success or failure. Patients were monitored, in some cases for >5 years after surgery, to compare their immediate postoperative examination with subsequent assessments to identify factors that predict long-term success as defined by the continued absence of defects at any site and to predict the need for subsequent surgery to repair defects at any site.

Material and methods

Between November 1, 1984, and October 31, 1990, 81 women underwent sacrospinous ligament suspen-

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6/6/37119

Table I. Previous pelvic surgeries in 58 of 81 women undergoing surgery for sacrospinous ligament suspension and pelvic reconstruction

Procedure	*Percentage of women*
Abdominal hysterectomy	42
Anterior-posterior repair	37
Vaginal hysterectomy	26
Retropubic urethropexy or needle suspension	25

sion and vaginal reconstruction for a variety of pelvic support defects. Before surgery each patient was objectively evaluated to assess the severity of support loss at five sites in the vagina: urethra, bladder, cervix or cuff, cul-de-sac, and rectum. All were examined in the dorsal lithotomy position, straining, and with the examining physician using a split speculum to visualize each site. Each site was scored on a scale of 0 to 4, with 0.0 indicating normal support. The cervix or vaginal cuff with 0.0 score is supported at or above the level of the ischial spines. The other sites have 0.0 score when they do not descend past the midvaginal axis, a plane extending from midhymen posteriorly to the apex, with straining. The scores of 1, 2, 3, or 4 are assessed when the site descends past the midvaginal axis halfway to the hymen (1), to the hymen (2), halfway out of the hymen (3), or fully outside the hymen (4). A pelvic support index is defined as the average score for all five sites in each patient. A patient with no defects has a pelvic support index of 0.0, and a patient with maximum loss of support at all five sites has a pelvic support index of 4.0. We tested the relationship between the 6-week postoperative pelvic support index and long-term success or lack of success in achieving anatomic restoration. If such a relationship exists, it can be used to identify the group of patients who are at an increased risk for further reconstructive surgery.

Results

Eighty-one women between 33 and 84 years of age (64 ± 11 years, mean ± SD) with pelvic support defects underwent sacrospinous ligament suspension and pelvic reconstruction. Previous pelvic surgery was documented in 72% of the patients (Table I). Preoperatively, all patients had at least one site protruding to or through the hymen. Fifty percent had ≥1 sites protruding halfway outside the hymen, and 38% had ≥1 sites protruding entirely outside the hymen. The percentage of patients with support loss at specific sites is shown in Table II. All patients required not only sacrospinous ligament suspension but a variety of other reconstructive procedures (Table III).

At the time of the first postoperative visit, 66 (81%) patients had no anatomic defects at any of the five vaginal sites (pelvic support index = 0.0), 14 (17%) patients had one or two sites protruding halfway to the hymen (pelvic support index > 0.0), and one (2%) had a defect to the hymen; however, none of the defects were larger than they had been preoperatively (Fig. 1). Eight of the 15 patients without normal support (pelvic support index > 0.0) had postoperative defects in bladder support, yet none had either a cystocele repair or retropubic urethropexy performed at the time of sacrospinous ligament suspension. Four of the 15 had cystocele repair and 3 had rectocele repair; however, in each case a defect persisted halfway to the hymen. Four of the 15 patients required further reconstructive surgery within 3 years of the original repair. One additional patient had a cystocele protruding halfway outside the hymen but she was too fragile for further surgery.

Thirteen patients with normal support (pelvic support index = 0.0) at the first postoperative visit later had anatomic defects at ≥1 sites. Five of the 13 patients who developed defects in bladder support had had no cystocele repair at the original surgery. Seven had had cystocele repair but subsequently had recurrent cystoceles. In only one case did the bladder protrude halfway outside the hymen, and that patient has had no progression in the size of the defect in 4½ years. One of these 13 patients has an enterocele to the hymen. None have had further surgery.

Forty-one of the 81 patients have had periodic pelvic exams for >2 postoperative years. Thirty of the 41 had

Table II. Percentage of 81 patients with each of five scores for support at each of five vaginal sites before surgery

	*Vaginal support scores**				
Vaginal site	*0*	*1*	*2*	*3*	*4*
Urethra	17	40	25	13	5
Bladder	6	10	19	33	32
Cervix-cuff	0	3	31	33	33
Cul-de-sac	15	15	23	32	15
Rectum	4	17	38	26	15

*Scores ranging from 0 with normal support and anatomic position to 4 with structure protruding fully outside the hymen.

Table III. Percentage of 81 patients receiving each operative procedure at surgery

Procedure	*Percentage of women*
Sacrospinous ligament suspension	100
Rectocele repair	95
Enterocele repair	73
Cystocele repair	70
Vaginal hysterectomy	32
Retropubic urethropexy	9

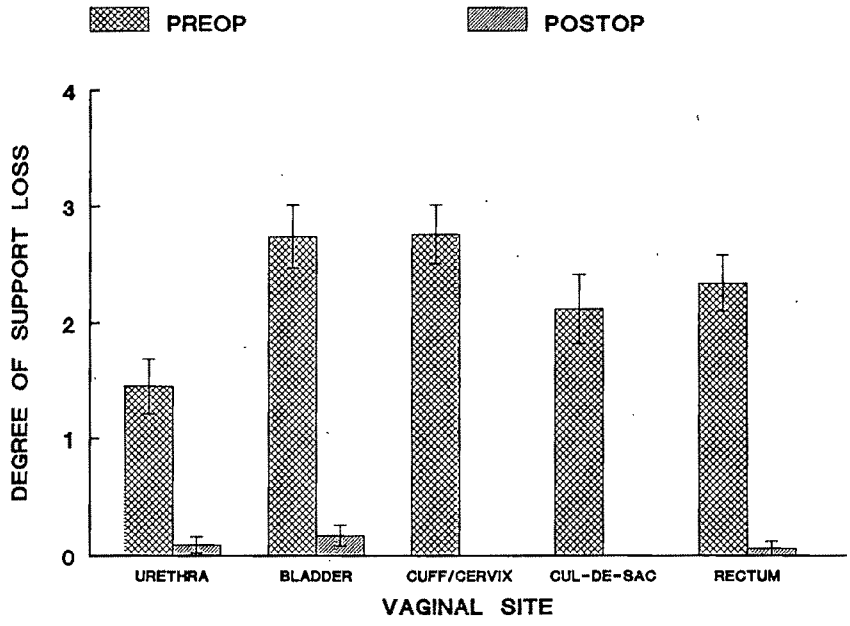


Fig. 1. Comparison of preoperative and postoperative scores for the degree of loss of support at each vaginal site in 81 patients. *Bars,* Mean and 95% confidence limits on average scores before surgery and at the first postoperative visit.

Table IV. Loss of pelvic support by 2 to 5 years after sacrospinous ligament suspension operation for patients with different pelvic support index at initial postoperative visit

Loss of support with tissue outside of the hymen by 2-5 years	*At first postoperative visit*	
	PSI = 0.0	*PSI > 0.0*
No	29	6
Yes	1	5*

PSI, pelvic support index.
*$p = 0.003$.

no defects at the first postoperative visit (pelvic support index = 0.0). Nine of those 30 subsequently developed defects at one or more sites over the next 2 to 5 years. Only one has a site extending outside the hymen.

The remaining 11 patients with any support defects (pelvic support index > 0.0) at the first postoperative visit have been monitored >2 years. Four of these 11 have had further surgery, and 1 other has tissue outside the hymen. Three had cystoceles, 1 a rectocele, and 1 a recurrence of her cuff prolapse.

The Fisher exact test showed that patients who have normal support (pelvic support index = 0.0) at the first postoperative visit are less likely to have tissue outside the hymen or to require surgery in the next 2 to 5 years than patients who have any support defect (pelvic support index > 0.0) at the first postoperative visit ($p = 0.003$) (Table IV).

Twenty-eight of the original patients demonstrated ≥1 defects at ≤5 years of surgery (Tables V and VI). Thirteen of the 28 have bladder support defects that were not repaired at the original procedure. Ten have had defects protruding to or through the hymen; four of those patients have had further surgery. The remaining 18 have no defects protruding to the hymen and are asymptomatic. All 18 have pelvic support indexes lower than their preoperative pelvic support indexes; however, they do not have full anatomic restoration. The principal site of support loss during the follow-up period was the bladder.

Comment

Pelvic reconstruction for vaginal prolapse is among the most challenging operative procedures in benign surgery. The surgeon must attempt to accomplish three separate but interrelated goals: restore anatomy (correct the anatomic defects), maintain or restore normal bowel and bladder function, and maintain vaginal capacity for sexual intercourse. Most authors report on their success in preventing cuff prolapse but do not objectively describe all the preoperative or postoperative anatomic defects. Consequently, analysis of factors that predict success or failure of anatomic restoration is impossible. Preoperative and postoperative analysis of visceral and sexual function is also important. We chose not to address these two parameters in our current report.

We have described a method of preoperative and postoperative assessment of five anatomic sites in the vaginal canal: urethra, bladder, cervix or cuff, cul-de-sac, and rectum. On the basis of our data, the patient

Table V. Pelvic support loss during postoperative follow-up period

Groups of patients		*n*	*%*
Receiving SSLS procedure		81	100
PSI = 0.0 postoperatively		53	66
Support defects at any site* (total)		28	34
Patients with support defects			
Grade 1	Halfway to hymen	18	22
Grade 2	To hymen	5†	6
Grade 3	Halfway outside hymen	5‡	6
Grade 4	Entirely outside hymen	0	0

SSLS, Sacrospinous ligament suspension; *PSI*, pelvic support index.
*For patients with loss of support at any site and at any time from 6 weeks to 5 years after surgery.
†One patient with support defect to level of hymen underwent repeat surgery.
‡Three patients with support defects outside hymen underwent repeat surgery.

returning for her first postoperative visit with no defects (pelvic support index = 0.0) has a 3% chance of having any defect to or through the hymen in ≤5 years. When she has any defect (pelvic support index > 0.0) at the first visit, she has a 45% chance of having a defect protrude outside the hymen in ≤5 years. These defects may require more surgery to relieve symptoms.

In our series 13 (16%) patients who first returned with defects or later had defects had poor preoperative support for the bladder (halfway to the hymen) but had no bladder repair performed during the original surgery. The surgeon either underestimated the severity of the defect on preoperative examination or believed that repair of the other defects was sufficient to minimize or correct the anterior defect. Bonney[6] addressed this problem when he discussed the potential effects of "retroversion of the vagina" in 1934. He suggested retroversion itself probably causes few symptoms; however, it is "a step towards prolapse of the anterior vaginal wall," opening the anterior cul-de-sac and allowing "abnormal pressure" to be exerted on the anterior vaginal wall and underlying fascia.[6] We have observed that patients with sacrospinous ligament suspension have an exaggerated posterior axis of the vagina, perhaps retroversion as described by Bonney. If his theory is accurate, we have predisposed all our patients to anterior defects in much the same way a high retropubic urethropexy predisposes a woman to defects in the posterior cul-de-sac. In the patients with anterior loss of support (cystocele), no matter how slight, we should consider repairing the bladder support to minimize the effects of "vaginal retroversion." Two of the four patients who required repeat surgery might have been spared the second operation if the first had included repair of the anterior segment.

The other 15 (18%) patients had correction of all defects that subsequently persisted or recurred. We can only speculate whether surgical technique, suture material, wound healing, tissue strength, or alteration in the vaginal axis played major roles in these patients. None have had further surgery. None have complaints with prolapse.

Table VI. Percentage of 28 patients with loss of pelvic support at each site and by each score during postsurgical follow-up period

	Vaginal support scores				
Vaginal site	*0*	*1*	*2*	*3*	*4*
Urethra	64	32	4	0	0
Bladder	7	57	18	18	0
Cuff	96	0	0	4	0
Cul-de-sac	92	0	4	4	0
Rectum	92	4	0	4	0

The prolapsed vaginal cuff was managed successfully in 98% of patients. Unfortunately, defects in bladder support were not managed as successfully. More accurate preoperative assessment of the anterior segment (bladder and urethra) should reduce the likelihood of missing a defect either in the office or in the operating room. We are currently evaluating the proper surgical technique to repair defective support for the bladder and urethra. Transvaginal reattachment of the periurethral and perivesical fascia to the tendinous arch appears to be the correct anatomic approach to consider.

Our data show that successful surgical management of the patient with anatomic support defects requires accurate preoperative and intraoperative identification of each defect and a specific reconstructive procedure to correct each defect. Sacrospinous ligament suspension is an important part of total pelvic reconstruction.

Oncologists stage tumors to compare results of different treatment modalities and to offer prognosis for cure or recurrence. Infertility surgeons stage endometriosis to compare results of therapy and offer prognosis for success. Unfortunately, pelvic reconstructive surgeons have not used an objective classification for

pelvic support defects. As a result, most reports on pelvic reconstruction compare apples to oranges, offering the serious reconstructive surgeon little objective information with which to plan an operation or to counsel a patient regarding prognosis. We hope that an objective, site-by-site, anatomic analysis of preoperative and postoperative physical findings will stimulate others to call for an approach to the staging of pelvic relaxation anatomic defects, allowing surgeons everywhere to communicate accurately with one another.

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Discussion

Dr. Sam P. Patterson, Memphis, Tennessee. I thank Dr. Shull and his coauthors for sending me their manuscript on sacrospinous ligament fixation and its revision in advance of this meeting. I also thank Dr. Podratz for asking me to discuss this paper and for allowing me to assume this podium for the first time since I had the honor of delivering the Presidential Address to this association in this room in 1983. I offered to defer to any younger volunteer; there apparently being none, here I am. Perhaps this could be because of the Cruikshank-Morley sparring episode of 1989.

I agree with the authors that pelvic reconstruction for prolapse is a challenging gynecologic procedure both historically and contemporaneously, as is evidenced by the multitude of techniques advocated throughout the years. As the ages of my referring physicians and my patients advance with my own, I see increasing numbers of women with recurrent severe degrees of prolapse and associated genital relaxations. Because of Drs. Morley and Cruikshank's enthusiastic advocacy of sacrospinous fixation for the prolapsed vaginal vault and discussions with David Nichols, I have performed or assisted with nine such procedures in the past year since our meeting in Louisville. Most authors attest to few complications, but there was a recent report of massive postoperative vaginal evisceration.[1] Thus far the incidence of recurrent vault prolapse after sacrospinous fixation seems small; but as years pass undoubtedly we shall see increasing numbers as the authors quote Dr. TeLinde's experience.[2] Others have had less than perfect results with vaginal repairs, or we would not have had modifications we associate with Heaney, McCall, Halban, Moschowitz, Kelly, Raz, Marshall, Machetti, Burch, Pereyra, Stamey, and Krantz. President Youngblood likewise has discussed disappointing results with surgical correction of stress urinary incontinence and has advocated Richardson's paravaginal defect repair.[3]

The authors' efforts to identify factors that predict long-term success are admirable and should be assessed for all surgical procedures. Numeric grading with scores of 1 to 4 for five sites is suggested, and a pelvic support index is defined as an average of these. Many numbers are reported in the tables, but the text refers only to a pelvic support index of 0 or >0. I ask Dr. Shull if a simple report of recurrence of cystocele, rectocele, enterocele, or prolapse might have made the results more understandable. The authors report that a patient with a pelvic support index of >0 on the first postoperative visit has a 45% chance of a defect protruding outside the hymen within five years. If accurate, this figure only includes those patients operated on during the first year of the study. If this figure includes patients operated on from 1987 on, 5 years have not lapsed, and that postoperative defect incidence would be higher.

I congratulate Dr. Shull et al. on their reported successful support of the vaginal cuff. In the comment the authors state that patients with a pelvic support index of 0 on the first postoperative visit have a 3% chance of having a defect to or through the hymen in ≤5 years. The data report one cystocele through the hymen and one enterocele to the hymen. These figures do not seem compatible. There were 41 patients monitored for >2 years; therefore 40 patients were monitored for <2 years. Restoration of visceral and sexual function is stated as a surgical goal, but these important physiologic functions are not addressed. Perhaps this should be considered a preliminary report, and subsequently we can be presented with larger numbers of women monitored for a longer time with appraisal of physiologic sexual and excretory function. I agree with their recommendation of an objective site-by-site preoperative and postoperative analysis of findings, but find their numeric averaging confusing, especially the repeated jumping from numbers to percentages in the text. Postoperative emphasis seems to be on specific site defects and not on pelvic support index, the original thrust of the paper. Objective descriptions of specific preoperative defects and reporting of postoperative results, both successes and failures, and recurrences would be more informative, particularly if addressed both anatomically and functionally.

Thanks to the authors for allowing me to participate in this discussion of fundamental gynecologic surgery.

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DR. LESTER A. BALLARD, JR., Cleveland, Ohio. Drs. Baden, Walker, and Shull from the Scott & White Clinic have had long and recognized interest in the classification of pelvic relaxation. Dr. Baden[1] made a significant attempt at classification in 1968, grading six vaginal areas: urethra, bladder, prolapse, cervix or vaginal cuff, cul-de-sac, rectum, and perineum. The strain at each site was graded from 0 to 4, depending on the degree of relaxation: 1 is halfway to the hymen, 2 is to the hymen, 3 is halfway past the hymen, and 4 is fully past the hymen. This classification allows individual preoperative and postoperative profiles to be compared.

Drs. Nagata and Kato[2] modified Dr. Baden's classification by deleting the perineum, leaving five areas and creating the urethrocele, cystocele, vaginal vault prolapse, enterocele, rectocele (UCPER) operative score. Each area is again graded from 0 to 4, and scores can still be compared before and after surgery.

Dr. Shull et al. use these same five areas and scores, but they average them to make the pelvic support index. They have applied this index in 81 cases to illustrate its use.

It is important that we adopt a common anatomic classification to prevent the many misunderstandings that have resulted from each author's perception of the classification. In 1989 at Central Association, I discussed Dr. Cruikshank's paper on sacrospinous fixation. I tried to point out the differences between his classification and that of Drs. Morley and DeLancey. There can be, and is, a certain distrust of another person's staging.

The ideal classification would include anatomic measures and symptoms to indicate the need for and type of surgery. However, Dr. Shull has based his index solely on anatomic defects, although there are patients without anatomic defects who have symptoms and patients with anatomic defects who have no symptoms. Although I agree that anatomic assessment must be done with straining in the lithotomy position, I am also impressed with how much more relaxation can be appreciated when the patient is examined while standing. Some urogynecologists grade the cystourethroceles by having the patient stand with a full bladder for a rest and stress voiding cystourethrogram to show maximum anterior relaxation.

In Dr. Shull's analysis of 81 sacrospinous ligament suspensions, 26 (32%) patients had the procedure done with a hysterectomy and 58 (68%) had the procedure after hysterectomy for vaginal cuff prolapse. In Table II, after converting the percentages to numbers of patients, I find 371 preoperative defects of various grades. Of a possible 405 defects, only 34 sites showed no defects. All 81 cases had defects of the cervix or cuff with grades 1 to 4 in addition to 67 urethra, 76 bladder, 68 cul-de-sac, and 78 rectal defects. Preoperatively, the anterior wall represented 38% of the total defects. In Table III 64 (79%) patients had cystocele repair or retropubic urethropexy, leaving 17 patients without anterior wall repair. There were 3 transient postoperative nerve injuries on the right side ascribed to "too high leg holders," not to surgical trauma. On which side were these 3 sacrospinous ligament suspensions done?

At the time of the first postoperative visit, 66 (81%) patients had pelvic support indexes of 0, but 15 (19%) patients had pelvic support indexes >0. Fourteen patients had grade 1 defects at ≥1 sites, but "none of these defects were larger than they were preoperatively." What were the grades preoperatively? Of these 15 patients with postoperative pelvic support indexes >0, eight had bladder defects that had no anterior wall repair. What were the preoperative grades of these eight patients? The other seven patients had surgical repair: four cystocele and three rectocele. Four of 15 defects required further reconstructive surgery, but from what preoperative group did they come? Thirteen patients who had pelvic support indexes of 0 at 6 postoperative weeks later had ≥1 defects. Five of these patients had no previous anterior repair but were scored 0 at 6 weeks, presumably with no defect present. In 7 patients who had cystocele repair, cystocele recurred: 6 grade 1 and 2, 1 grade 3, and 1 grade 2 enterocele. When comparing 41 of 81 patients monitored for >2 years, there were 30 whose pelvic support index was 0 at first postoperative check. Nine had defects, with 8 grade 1 or 2 and 1 grade 3. The 11 patients with pelvic support indexes >0 had 1 cuff prolapse, 3 cystoceles and 1 rectocele, with 4 subsequently requiring surgery. Therefore 1 (3%) of 30 pelvic support index = 0 had a grade 3 defect, and 5 of 11 pelvic support indexes > 0 (45%) had grade 3 defects.

Dr. Shull combined all of the 28 (34%) postoperative defect cases without regard to pelvic support index or length of follow-up. Thirteen patients had no initial bladder repair, which has been previously noted: five from the pelvic support index = 0 group and eight from the pelvic support index > 0 group. Ten of these patients had grade 2 and 3 defects, and 4 of these had surgery. The remaining 18 patients with grade 1 defects were asymptomatic.

Table VI shows 41 postoperative defects. Of these, 10 were of the urethra and 26 were of the bladder; that is, 36 (87%) of 41 were anterior defects. This compares with 38% for preoperative anterior defects. The other postoperative defects show 1 grade 3 vault, two grade 2 and 3 cul-de-sacs, and 2 grade 1 and 2 rectal defects.

Dr. Shull has shown that whether anterior defects are repaired or not, the anterior wall is the area in which defects develop. Dr. Nichols[3] recently stated in regard to sacrospinous ligament suspension that "full-length

anterior colporrhaphy with special attention to support of the cystourethral junction is performed almost without exception." I agree that the anterior wall is the major defect with sacrospinous ligament suspension. In our evaluation of 110 sacrospinous ligament suspension patients at Cleveland Clinic from 1978 through 1985, it was apparent that the anterior wall was a problem. Although we had 3 vault recurrences, we had 5 grade 2 cystoceles, 3 with stress urinary incontinence and 8 with transitory stress incontinence. I thought at this time that I needed to do better anterior repair with either pubourethral suspension or with the retropubic or retrovaginal needle procedure. However, when I reviewed our next 158 sacrospinous ligament suspension patients from 1986 through 1989, it was apparent that we had not improved the anterior wall defects and stress incontinence in spite of a diligent preoperative attempt to sort out anatomic and sphincter incontinence and bladder instability. On evaluation of these 158 sacrospinous ligament suspension patients we had 3 grade 3 vault prolapses but had 2 grade 2 anterior enteroceles, 10 grade 2 cystoceles, 1 grade 3 cystocele and 6 with stress incontinence. I became suspicious that the sacrospinous ligament suspension operation was contributing to the anterior wall defects.

Dr. A. Cullen Richardson et al.[4] has pointed out that when we pull the vault either too far anteriorly or too far posteriorly we produce a compensatory abnormality. It will be interesting to know if a paravaginal repair of the anterior wall in patients with sacrospinous ligament suspension, which Dr. Shull presented to the Central Association in 1988, will decrease these defects. Will doing a perirectal vault suspension along with paravaginal suspension improve the anterior wall? In 237 of the 310 sacrospinous ligament suspension I have done, my enthusiasm for the procedure has been tempered because of the defects of the anterior wall.

I applaud Dr. Shull's efforts to create the pelvic support index, but I do not think it is as helpful as the preoperative and postoperative profiles of each defect. The index reduces specificity because it replaces five numbers with one, and it does not provide any corresponding increase in use. It seems to me that clinical decisions still have to be made on the basis of a profile and not on the index. I guess I am a splitter rather than a lumper. However, I believe that Dr. Shull and his group have stimulated us all by calling for, as he says in the last sentence of his manuscript, "an approach to the staging of pelvic relaxation anatomic defects, allowing surgeons everywhere to communicate accurately with one another."

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Dr. Mickey Karram, Cincinnati, Ohio. I would like to ask Dr. Shull how he evaluated women who were initially seen with primary complaints of lower urinary tract dysfunction, whether it be voiding dysfunction or urinary incontinence. Did these patients undergo urodynamic evaluation?

In your series there was a small group of patients who underwent retropubic procedures in conjunction with vault suspension. However, most of the patients just underwent standard anterior repair. How did you decide which operation to do?

Last, what is the best way to test preoperatively for potential stress incontinence in patients with massive pelvic relaxation?

Dr. G. Rodney Meeks, Jackson, Mississippi. I rarely see a patient without an anterior defect who needs a cuff suspension. I wonder why one third of your patients fell into this catagory.

Second, can you overcorrect the vaginal defect by pulling the vagina too far back toward the ligament? Is there a depth that you try to achieve when you put the suture in, or do you try to pull the vaginal cuff as far back as possible?

Dr. Jeffery Cornella, Scottsdale, Arizona. It is also our impression that sacrospinous fixation increases the incidence of subsequent anterior wall defect, and we support the concept of prophylaxis. However, in the last number of years I have noted increased case reports documenting bowel eviscerations through the vagina related to concomitant needle suspension and sacrospinous suspension, even though previously in the world literature there have been <39 reports associated with gynecologic surgery.

Would Dr. Shull address whether this combination of procedures weakens the apex, how he tempers his prophylaxis regarding the apex of the vault, and how he approaches patients who have decreased thickness of the apical tissues?

What number and type of sutures does Dr. Shull use, and how many bites are taken into the vagina and ligament?

Dr. Stephen H. Cruikshank, Minneapolis, Minnesota. Why does Dr. Shull not examine these patients in the standing position; this does test the anatomic support to the *n*th degree because that is how humans usually are, in the standing position.

At the time of hysterectomy do you evaluate these people, and have you done it preoperatively both in the supine and the erect position so that you can do the right procedure?

Third, I think that we should understand the definition of prophylaxis before you answer that, and that means to prevent or to ward off. I looked that up in Webster's dictionary before I came this year.

Dr. Shull (Closing). Dr. Patterson said it would be simpler if there were not so many numbers. He is exactly right. I fretted over my manuscript and asked

other people to help with that concern; however, there was no way for us to know what the outcome would be without collecting the information. To begin with fewer observations would have been to reinvent a less specific description just as Dr. Clayton Beacham reported, that Dr. Cruikshank had in his paper, and that Dr. Morley used in his. We did not choose to start with something simple because we wanted to understand more about the problems of support defects and their repair. To relay that information to you it is important to give numbers. We tried to make our conclusions more concise by referring to patients who had abnormal support or normal support. If you believe it is important to communicate about pelvic support defects in a manner similar to staging, you know that the descriptions must be detailed, reproducible, and accurate in the same way that definite findings are required to stage genital cancers. I agree with both commenters that the pelvic support index may be confusing; however, collection and analysis of the information appear to be important.

Dr. Ballard suggested that we not only should have anatomic information but also symptomatic information. The purpose of our study, however, was to look at anatomy, not visceral function and not sexual function.

Dr. Nichols, who has written extensively about his surgical experience with sacrospinous ligament suspension, has not given detailed comments regarding follow-up of other areas of the vaginal canal. I do not know or presume to say what type of success he has with areas other than the cuff.

Dr. Ballard also asked about some of the information in the tables. I will look that up privately.

Dr. Mickey Karram asked about lower urinary tract dysfunction, why we chose to do a retropubic or a paravaginal repair, and what the test of choice is. The test of choice for me is a provoked full-bladder stress test when the apex of the vagina is replaced. We filled the bladder to the point of maximum sensation, reduced the apex with a sponge stick or a cotton swab, and had the patient cough and strain while sitting in a birthing chair. If the patient leaks urine under those circumstances, we choose vaginal or retropubic paravaginal defect repair in association with the other reconstructive procedures.

Dr. Meeks asked why we did not do an anterior repair. First, in some patients we did not make the right diagnosis. I am sure we missed the defects. Second, in some of the people we identified the defects preoperatively, but when we replaced the apex to the ischial spine (performed in the office) and had the patient bear down maximally, it was our impression the anterior segment did not descend. We were wrong.

Do we go to the spine? We try to take the apex of the vagina to the sacrospinous ligament. If that is not possible we use levator fascia just anterior to the spine, a procedure Dr. Meeks is very familiar with. The procedure was reported in *Southern Medical Journal* in 1963 by Byron Inman. The group at Mississippi has used that repair extensively. I have another group of patients I did not report today in whom I have used levator fascia as a point of attachment.

Jeff Cornella has asked about suture material. We have used permanent sutures in the right ligament, Novafil or Ethibond. There was not enough information for me to report whether it made a difference as to which we used. Steve Cruikshank asked if we examined patients when they were standing. Yes and no. I do examine some standing patients. I find it quite difficult to look at each site with the patient in the standing position. I can not fathom being able to do that accurately. I could tell if something were coming out, but it would be quite difficult to evaluate urethra, bladder, cuff, cul-de-sac, and rectum individually with the patient in the standing position.

Cervical cytology: A randomized comparison of four sampling methods

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OBJECTIVE: The purpose of this study was to compare smear quality and endocervical cell recovery of four cervical smear sampling devices.

STUDY DESIGN: Two thousand fifteen patients undergoing routine cervical smears at the University of Tennessee Obstetrics and Gynecology clinics were randomly assigned to a cotton swab-spatula, Cytobrush-spatula, Cervex-Brush or Bayne Pap Brush. The cytopathology laboratory, blind to method, used specific criteria to grade smears as being optimal, adequate, marginal, or inadequate. Statistical analysis was by the χ^2 and analysis of variance tests.

RESULTS: No statistical differences occurred among the groups for nonpregnant patients. For pregnant patients smear quality was improved with both Cytobrush-spatula and Bayne Pap Brush versus cotton swab-spatula ($p = 0.0301$ and 0.0004, respectively); cotton swab-spatula had fewer endocervical cells than the Cytobrush-spatula ($p = 0.0001$), Cervex-Brush ($p = 0.0286$), and Bayne Pap Brush ($p = 0.0081$).

CONCLUSIONS: The cotton swab-spatula and Cytobrush-spatula appear to be the most effective screening methods for nonpregnant and pregnant patients, respectively. (Am J Obstet Gynecol 1992;166:1772-9.)

Key words: Papanicolaou smear, cervical smear, Cytobrush, Cervex-Brush, Bayne Pap Brush

Routine cytologic screening for cervical dysplasia has effectively decreased the incidence of cervical carcinoma and its associated mortality[1]; however, the false-negative rate for Papanicolaou smears is estimated to be as high as 40%.[2] Suboptimal smear quality is a known potential source for such error. Because the squamocolumnar junction is at greatest risk of having neoplasia develop, adequate cell sampling of this area is necessary to assure the high sensitivity of the Papanicolaou smear. The presence of columnar endocervical cells on the smear is accepted as evidence that the squamocolumnar junction has been sampled. Large studies have shown that the presence of endocervical cells is associated with an increase in the proportion of smears showing cellular atypia and dysplasia, therefore resulting in higher sensitivity.[3-6]

To improve Papanicolaou smear quality and reliability, numerous cell collection devices and techniques have been introduced since the original method described by Papanicolaou in the 1940s.[7-11] The ideal instrument would provide adequate ectocervical and endocervical cellular material without undue mucosal damage to the cervix and without inducing bleeding that could obscure cytologic evaluation. The technique should be relatively simple and produce similar quality Papanicolaou smears regardless of the experience of the medical personnel collecting the samples. Therefore the purpose of this study was to compare four commercially available Papanicolaou smear–collecting devices to determine which method produces the highest quality smear in pregnant and nonpregnant patients.

Material and methods

The study group included all patients undergoing Papanicolaou smear evaluation between October 1990 and February 1991. Subjects were evaluated in the outpatient clinics of the Regional Medical Center in Memphis, the teaching hospital associated with the University of Tennessee, Memphis. Papanicolaou smears were performed by faculty, residents, and medical students, all of whom had been instructed in the proper technique of specimen collection with each device. Only patients who had previously undergone hysterectomy or who had previously been treated for cervical carcinoma were excluded. Patients were not excluded because of their gestational status.

Demographic data obtained at the time of the cervical

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smear included age, gravidity, parity, gestational status, date of last menses, current use of oral contraceptive, history of previous abnormal Papanicolaou smear, and previous cervical cryotherapy, cervical conization, or cervical laser treatment. Gestational status was defined as (1) pregnant, any gestational age; (2) postpartum, <8 weeks from delivery of pregnancy >20 weeks' gestational age; and (3) nonpregnant. The training level of the person taking the smear was also recorded.

Patients were randomly assigned by computer-generated numbers to one of four methods of Papanicolaou smear collection. The four cytologic sampling methods evaluated were (1) cotton swab and modified Ayre spatula, (2) Cytobrush (Medscand (U.S.A.), Hollywood, Fla.) and modified Ayre spatula, (3) Cervex-Brush (Unimar, Inc., Wilton, Conn.), or (4) Bayne Pap Brush (Medical Dynamics Inc., Englewood, Colo.) (Fig. 1). The Papanicolaou smear collection method was per the manufacturer's recommended instructions. Each method was preceded by use of the modified Ayre spatula, which was rotated circumferentially over the ectocervix; the end appropriate for the patient's gestational status was used. The cotton swab and Cytobrush were each gently inserted into the endocervical canal and rotated 360 degrees. The central bristles of the Cervex-Brush were gently inserted into the endocervix until the lateral bristles bent fully against the ectocervix. The Cervex-Brush was rotated three to five times to the right and left. The sample as applied to the slide with a paint stroke of each side of the bristles. The Bayne Pap Brush is malleable and can be adjusted to conform to the shape of the cervix. The endocervical portion of the brush was gently inserted into the endocervical canal until there was continuous application of the brush from the endocervix to the ectocervix. The brush was rotated 360 degrees. All smears were placed on a single glass slide and immediately fixed with 95% alcohol.

All smears were evaluated by the Cytopathology Laboratory associated with the Department of Pathology, University of Tennessee, Memphis. The laboratory is accredited by The College of American Pathologists. The cytopathology technicians and cytopathologist were blind as to the sampling method. Initially one of four cytopathology technicians interpreted the slides. The cytopathologist reviewed any sample about which the cytotechnician was uncertain, 200 randomly selected samples, and all samples that were deemed unsatisfactory or abnormal. Recovery of endocervical cells and quality of the smear were documented. The presence of inflammation, bacteria, *Monilia, Trichomonas* excessive red blood cells, and drying effect were noted.

Smear quality was designated as inadequate, marginal, adequate, or optimal. A smear was considered inadequate if (1) it lacked an endocervical component, (2) excess blood or inflammation obscured the epithelial cells, (3) the smear dried before fixation. (4) the ectocervical component was insufficient, or (5) the smear was too thick. A smear were graded as marginal if the quality was compromised, but not obscured, by blood, inflammation, or air-drying; in addition, ectocervical cells were present, and the smear contained ≥25 endocervical cells. The quality of adequate and optimal smears was uncompromised by the above-noted factors. Ectocervical cells were present. Adequate smears contained ≥25 but <50 endocervical cells. Optimal smears contained ≥50 endocervical cells. If the smear were deemed inadequate or marginal, the reason was recorded. Statistical analysis was performed by the Department of Biostatistics. Analysis of variance and χ^2 tests were used.

Results

A total of 2015 patients were randomized to be tested with one of four cervical cytologic sampling devices: 503 (25.0%) cotton swab and modified Ayre spatula, 501 (24.9%) Cytobrush and modified Ayre spatula, 505 (25.0%) Cervex-Brush, and 506 (25.1%) Bayne Pap Brush. Table I summarizes the demographic data of the patients included in the clinical trial. There was no statistical difference among the four sampling groups regarding age, gravidity, parity, gestational status, oral contraceptive use, postmenopausal satus, and training level of the examiner. The number of patients in each group with histories of previous abnormal Papanicolaou smears or cervical surgery, including cryotherapy, laser ablation, or conization, was not statistically different.

There was a significant difference in Papanicolaou smear quality between pregnant and nonpregnant patients (postpartum patients were too few for statistical evaluation). Five hundred two (85.4%) of 588 nonpregnant patients had optimal-adequate smears, compared with 1004 (74%) of the 1356 pregnant patients ($p < 0.001$). Inadequate smears occurred in 67 (11.4%) of the 588 nonpregnant patients and 242 (17.8%) of the 1356 pregnant patients ($p = 0.0050$). Endocervical cells were absent in 164 (12.1%) of the 1356 pregnant patients, compared with 48 (8.2%) of the 588 nonpregnant patients ($p = 0.0220$). Excessive inflammation was found on cervical smears of 212 (15.6%) of the 1356 pregnant patients and 72 (12.2%) of the 1588 nonpregnant patients ($p < 0.0001$). There was no statistical difference between groups for excessive blood.

Cervical smear quality for each sampling device was assessed separately for nonpregnant-postpartum and pregnant patients. Because both optimal and adequate smear grades are acceptable to the practitioner, these were combined into one category for statistical comparison. For nonpregnant and postpartum patients

Table I. Comparison of demographic data and training level of the health care professional obtaining Papanicolaou smear

	Cotton swab–modified Ayre spatula (n = 503)		*Cytobrush–modified Ayre spatula (n = 501)*		*Cervex-Brush (n = 505)*		*Bayne Pap Brush (n = 506)*		
	No.	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*p Value*
Age (yr, mean ± SD)	24.1 ± 7.05		24.8 ± 7.3		25.0 ± 8.7		25.0 ± 10.1		NS
Gravidity (mean ± SD)	2.7 ± 1.8		2.6 ± 1.7		2.6 ± 1.7		2.7 ± 1.9		NS
Parity (mean ± SD)	1.5 ± 1.6		1.4 ± 1.4		1.5 ± 1.5		1.6 ± 1.7		NS
Nonpregnant	140	27.8	141	28.1	149	29.5	158	31.2	NS
Pregnant	345	68.6	340	67.9	340	67.3	331	65.4	NS
Postpartum (≤8 wk)	18	3.6	20	4.0	16	3.2	17	3.4	NS
Oral contraceptive use	58	11.5	49	9.8	47	9.3	54	10.7	NS
Postmenopausal	4	0.8	2	0.4	9	1.8	8	1.6	NS
Previous abnormal Papanicolaou smear	45	8.9	44	8.8	62	12.8	60	11.8	NS
Previous therapy									
Previous cryotherapy	12	2.4	9	1.8	15	3.0	17	3.4	NS
Previous conization	5	1.0	7	1.4	11	2.2	8	1.6	NS
Previous laser ablation	4	0.8	3	0.6	5	1.0	1	0.2	NS
Level of training									
Medical student (n = 687, mean 34.1%)	167	33.2	176	35.1	177	35.0	167	33.0	NS
PGY-1 (n = 271, mean 13.4%)	54	10.7	61	12.2	79	15.6	77	15.2	NS
PGY-2 (n = 384, mean 19.0%)	102	20.3	99	19.8	95	18.8	88	17.4	NS
PGY-3 (n = 261, mean 13.0%)	70	13.9	72	14.4	50	9.9	69	13.6	NS
PGY-4 (n = 76, mean 3.8%)	30	6.0	25	5.0	26	5.1	25	4.9	NS
Fellow-attending (n = 19, mean 0.9%)	3	0.6	4	0.8	7	1.4	5	1.0	NS
Not documented (n = 287, mean 14.2%)	77	15.3	64	12.8	71	14.1	75	14.8	NS

$p < 0.05$ considered significant. *NS*, Not significant; *PGY*, postgraduate year.

there was no statistical difference in smear quality among the four sampling groups (Table II).

The smear quality results of the 1356 pregnant patients sampled are listed in Table III. Optimal and adequate Papanicolaou smears were increased for the Cytobrush-spatula and the Bayne Pap Brush versus the cotton swab–spatula ($p = 0.0301$ and 0.0004, respectively). Fewer inadequate smears occurred with the Cytobrush-spatula versus the cotton swab–spatula ($p = 0.0317$), the Bayne Pap Brush versus the cotton swab–spatula ($p < 0.0001$), and the Bayne Pap Brush versus the Cervex-Brush ($p = 0.0077$). There were no other statistical differences occurring among sampling devices.

Factors accounting for marginal and inadequate smears for all patients are summarized in Table IV. Absent endocervical cells was the single most common reason for the Papanicolaou smears to be judged inadequate. In the pregnant patient the cotton swab–spatula was less likely to recover endocervical cells than the Cytobrush-spatula ($p = 0.0010$), the Cervex-Brush ($p = 0.0137$), and the Bayne Pap Brush ($p = 0.0026$). There was no differences among the other three sampling devices. This trend was evident, but not statistically significant, for the nonpregnant patient. For all patients excessive blood was more common in the Cytobrush–Ayre spatula smear as compared with the cotton swab–Ayre spatula ($p = ?$) and the Cervex-Brush ($p = ?$). No statistical difference occurred between the sampling methods for excessive drying or excessive inflammation. Smears that were too thick or too thin occurred too infrequently to be statistically meaningful.

Detection of cervical dysplasia was constant for all Papanicolaou smear sampling groups. Dysplasia was present in 31 (6.2%) cotton swab–spatula smears, 29 (5.8%) Cytobrush-spatula smears, 31 (6.1%) Cervex-Brush smears, and 27 (5.3%) Bayne Pap Brush smears.

Thirty-six patients underwent colposcopic examinations and biopsies at the time their Papanicolaou smears were obtained. Eight (22.2%) patients had clumping of cervical cells on their smears; the outer third to half of the epithelial layer of their biopsy spec-

Table II. A comparison of pap smear quality by sampling method in nonpregnant and postpartum patients

	Cotton swab (n = 158)		*Cytobrush (n = 161)*		*Cervex-Brush (n = 165)*		*Bayne Pap Brush (n = 175)*		
	No.	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*p Value*
Optimal and adequate	132	83.5	136	84.5	140	84.5	153	87.4	NS
Marginal	5	3.2	10	6.2	5	3.0	3	1.7	NS
Inadequate	21	3.3	15	9.3	20	12.1	19	10.9	NS

$p < 0.05$ considered significant. *NS*, Not significant.

Table III. A comparison of pap smear quality by sampling method in pregnant patients

	I Cotton swab (n = 345)		*II Cytobrush (n = 340)*		*III Cervex-Brush (n = 340)*		*IV Bayne Pap Brush (n = 331)*		
	No.	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*p Value*
Optimal and adequate	224	64.9	264	77.6	239	70.3	277	83.7	II vs I $p = 0.0301$ IV vs I $p = 0.0004$
Marginal	31	9.0	26	7.6	37	10.9	16	4.8	NS
Inadequate	90	26.1	50	14.7	64	18.8	38	11.5	II vs I $p = 0.0317$ IV vs I $p < 0.0001$ IV vs III $p = 0.0077$

$p < 0.05$ considered significant. *NS*, Not significant.

imens had been denuded. As a result, evaluation of cervical intraepithelial neoplasia grade for either the smear or the biopsy was impossible. One (12.5%) was obtained by the cotton swab–spatula, 2 (25.0%) by the Cytobrush-spatula, 4 (50.0%) by the Cervex-Brush, and 1 (12.5%) by the Bayne Pap Brush.

Comment

We believe this is the first report comparing the four commonly used sampling methods in a randomized trial in the same population, and thus the major strength of this study. Taylor et al.[7] reported a decline of suboptimal smears caused by a lack of endocervical cells from 12% to 1.7% when the Cytobrush was used in place of the cotton swab. Murata et al.[8] corroborated these findings and improved the endocervical cell yield from 61.8% to 82% by replacing the cotton swab with the Cytobrush. Concurrently they noted an increase in the proportion of Papanicolaou smears showing cervical intraepithelial neoplasia.

The Cervex-Brush was designed to sample the ectocervix and endocervix simultaneously. Vooijs[9] compared with Cervex-Brush to the modified Ayre spatula in a large series. The rate of unsatisfactory smears fell from 4.8% to 2.4%, and the presence of endocervical cells rose from 85.9% to 95.8% with use of the Cervex Brush. Laverty et al.[10] showed a statistically significant increase in endocervical cell recovery and presence of atypical squamous cells with the Cervex-Brush. However, both of these studies lacked the use of an endocervical sampling device in conjunction with the spatula in the comparison group.

In a prospective randomized trial Dotters et al.[11] reported a very low incidence of Bayne Pap Brush smears lacking endocervical cells (1.4%); 96% of these smears were optimal (containing >50 endocervical cells). Of the smears collected with the cotton swab and Ayre spatula, 19% lacked endocervical cells and only 58% were considered optimal. Dotters et al. also noted a 20% incidence of cervical bleeding when the Bayne Pap Brush was used in pregnancy and a 2.5% incidence of bleeding in nongravid patients.

This trial showed a significant improvement in smear quality for the Cytobrush over the cotton swab for pregnant patients. Frequency of inadequate smears was significantly less compared with the cotton swab–modified Ayre spatula method. Endocervical cell presence was significantly greater in the pregnant population. These differences were not statistically significant in the nonpregnant patient.

The Cervex-Brush did not demonstrate a significant change in smear quality as compared with the other groups. Endocervical cell presence was significantly improved over the cotton swab–spatula for pregnant patients. Excessive blood was seen more commonly with the Cervex-Brush than with the cotton swab–spatula.

Our study demonstrated a statistically significant improvement in smear quality for the Bayne Pap Brush as compared with both the cotton swab–spatula and the Cervex-Brush for pregnant patients. For nonpreg-

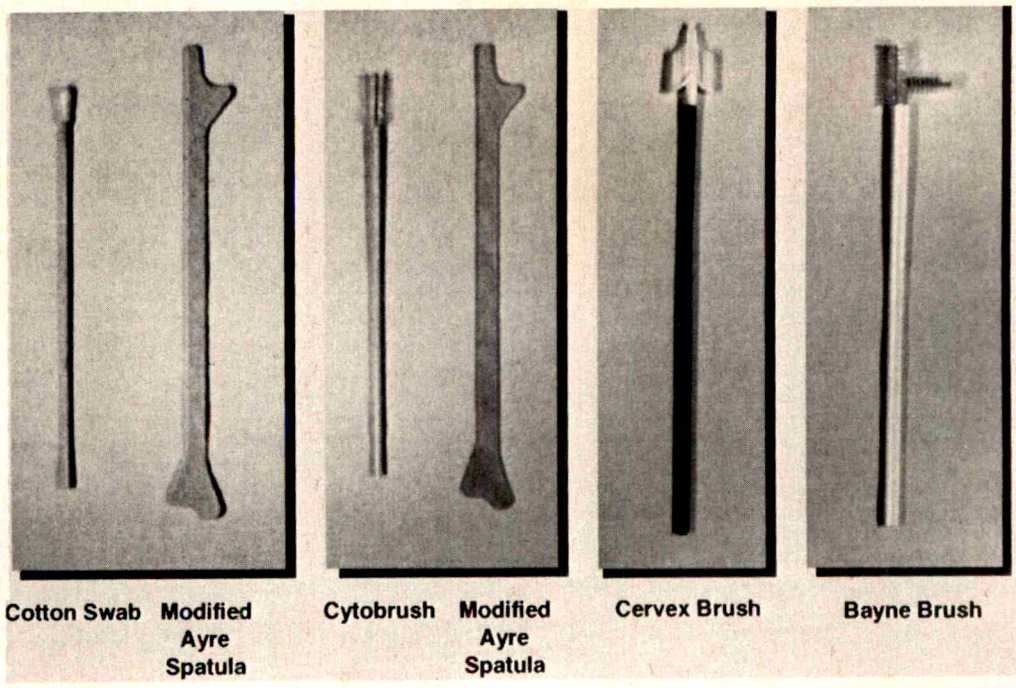


Fig. 1. Four methods of cervical cytologic sampling: Modified Ayre spatula, Cytobrush, Cervex-Brush, and Bayne Pap Brush.

Table IV. Factors responsible for marginal or inadequate smear quality

	I Cotton swab–modified Ayre spatula (n = 503)		*II Cytobrush–modified Ayre spatula (n = 501)*		*III Cervex-Brush (n = 505)*		*IV Bayne Pap Brush (n = 506)*		
	No.	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*p Value*
Endocervical cells absent (all patients)	83	16.5	36	7.2	51	10.1	47	9.3	II vs I $p = 0.0001$ III vs I $p = 0.0288$ IV vs I $p = 0.0081$
Excessive blood	13	2.6	38	7.6	37	7.3	17	3.4	II vs I $p = 0.0001$ III vs I $p = 0.0002$
Excessive drying	43	8.5	25	5.0	28	5.5	8	1.6	NS
Excessive inflammation	9	1.8	15	3.0	13	2.6	6	1.2	NS

$p < 0.05$ considered significant. *NS*, Not significant.

nant patients the Bayne Pap Brush was equal to the cotton swab–spatula. Endocervical cell collection was significantly improved over the cotton swab–spatula for pregnant patients.

A concern raised but not answered in our paper is the effect of the various sampling methods on the epithelium of the cervix. As seen in eight patients undergoing colposcopies, denudation of the epithelium by the sampling techniques made histologic evaluation of the biopsy impossible. Improved cell smears may be counterproductive if cervical intraepithelial neoplasia grade and depth of involvement cannot be assessed by biopsy, thereby mandating conization. As increasingly aggressive techniques are used to sample the endocervix, this issue will demand further study.

Cost is important when mass screening is considered. The manufacturers' quoted price (as of April 1991) per unit for 1000 units is $0.07 of the cotton swab–Ayre spatula, $0.30 for the Cytobrush–Ayre spatula, $0.41 for the Cervex-Brush, and $0.53 for the Bayne Pap Brush. The cost of greater quantities may vary, but the price trend is still applicable.

On the basis of these data, the less costly method of screening is the Ayre spatula–Cytobrush combination

in the pregnant patient and the Ayre spatula–cotton swab combination in the nonpregnant patient. If only one method is to be selected, it should be the Ayre spatula–Cytobrush method.

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Discussion

DR. JOHN V. KNAUS, Evanston, Illinois. In an era that has seen the Papanicolaou smear extrapolated from simple exfoliative cytology to reinvent on by the Bethesda system, Dr. McCord et al. have helped resolve several issues of daily importance to the practicing gynecologist.

First, in nonpregnant patients no significant statistical difference in Papanicolaou smear quality was noted among the four sampling methods tested. Cotton swab and spatula was judged the most cost- and smear-quality effective. In the pregnant patient the Cytobrush and spatula were most effective. Second, the detection rate of cervical intraepithelial neoplasia was equal among the four methods. As the authors state, no prior study has led to these clear results.

It is a third area, that of Papanicolaou smear adequacy as determined by the presence of endocervical cells, that requires further discussion. Of late much has been written expounding the close correlation between the presence of endocervical cells and Papanicolaou smear adequacy. Literature documenting this relationship has become an assumption of many authors. Indeed, an entire body of knowledge placing this supposition in doubt has been largely ignored. With the criteria of absent or insufficient endocervical cells, up to 30% of Papanicolaou smears may be inadequate.[1] Husain et al.[2] report this number to approach 80% in some series. In the case of a wide transformation zone, the most representative sampling may not contain endocervical cells.[3] Lee et al.[4] report endocervical cell recovery on cervical cytology smears to be independent of the clinician's length of training and experience. In a prospective study of 18,900 patients, Kivlahan found the presence of endocervial cells on Papanicolaou smears to have no statistical predictive value in detecting cervical intraepithelial neoplasia.[5]

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DR. JULIAN SCHINK, Chicago, Illinois. The stated goal of Dr. McCord's study was to compare the Papanicolaou smear quality and endocervical cell recovery of four different cervical sampling systems. Her study focuses on easily measured subjective parameters, but with this study design she is unable to address the most important question: Can a given Papanicolaou smear collection system improve the sensitivity of this screening test? This study could not use Papanicolaou smear sensitivity as an endpoint for two reasons. First, the prevalence of cervical dysplasia was too low in the study population. Second, the sample size is much to small to find any difference in the dysplasia detection rate of different systems. A study of Papanicolaou smear screening would require 26,500 patients per group to demonstrate a 10% difference in detection rate at a power of 80% and $p = 0.05$. With the present study the power to detect such a difference was only 9%.

For Dr. McCord's research to be meaningful we must accept the premise that an adequate sample of endocervical cells results in a more sensitive and reliable Papanicolaou smear. In support of this premise, Rylander[1] (1977) showed that false negative Papanicolaou smears of women with invasive cancer frequently lacked endocervical cells. Furthermore, Elias et al.[2] noted severe abnormalities four times more often in smears containing endocervical cells than in smears without endocervical cells.[2] On the other hand, however, in a study of 18,914 women Kivlahan and Ingram[3] found no difference in the atypia rates between women with and without endocervical cells on serial Papanicolaou smears. Therefore the importance of endocer-

vical cells on a Papanicolaou smear remains controversial. Many prior studies have shown the same improved yield of endocervical cells with the Cytobrush, but I do not know of any large prospective study showing improved sensitivity for detection of cervical cancer of dysplasia with the use of the Cytobrush. We can safely conclude that the Cytobrush produces a cytology specimen that is more likely to contain endocervical cells; it remains to be shown whether this translates into a more sensitive test.

In this study the improvement in Papanicolaou smear quality was most noteworthy in pregnant patients. Unfortunately, the Cytobrush manufacturer cautions against the use of Cytobrush in pregnancy. My questions to Dr. McCord are (1) How do the authors reconcile this discrepancy between their results in pregnant patients and the manufacturers' recommendation? and (2) Because almost 15% of your patients have unsatisfactory smears, how do you recommend managing these patients?

The power analysis was performed by Alfred W. Rademaker, Biometry Section of Northwestern University Cancer Center. This analysis is based on a two-tailed test detecting a 10% difference ($\leq$5.53% or $\geq$6.82%) in the baseline rate of 6.2%.

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Dr. John C. Morrison, Jackson, Mississippi. We in Mississippi have the same problem as Dr. McCord et al. have in Tennessee: the pregnant patient with an inadequate Papanicolaou smear that causes unnecessary patient visits. This adds greatly to the expense and to the uncertainty regarding the results.

Dr. Schink correctly points out that the manufacturer's guidelines state that the Cytobrush should not be used in pregnant patients. We have just completed a study involving 880 patients comparing Cytobrush with the cotton-tip applicator. We found that in 71% of the women the Cytobrush gave us adequate Papanicolaou smears compared with only 42% with the cotton tipped applicator, very similar to the statistics that Dr. McCord presented.

In our series of 880 patients only three had bleeding. We had a few cases, of course, with spotting. So we have no case in our series where the Cytobrush led to bleeding that was in any way dangerous; we are publishing that fact and ask the authors how many of their pregnant patients had bleeding that was heavy or caused complications.

Dr. James G. Blythe, St. Louis, Missouri. The first pictures you showed—was that a wooden or a plastic Ayre spatula? There is a statistically significant difference when collecting cells with the plastic Ayre spatula, and I certainly recommend that spatula to everybody.

We did a similar study comparing the Cytobrush with the Cervex-Brush, looking at the number of endocervical cells. We found endocervical cells to be equally as recoverable with the Cytobrush and the Cervex-Brush whether the patient was pregnant or not because the Cytobrush is not recommended for use in pregnancy. We recommend to our private physicians the use of the Cervex-Brush and the plastic Ayre spatula in pregnant patients.

Dr. Suzanne R. Trupin, Champaign, Illinois. In our practice we have been searching diligently for an affordable Papanicolaou smear, and we have switched labs a number of times over the past number of years. We find that our percentage of cervical intraepithelial neoplasia discovery is a factor of the individual pathology laboratory, as is our percentage of adequacy. Could the authors comment on this?

Dr. John Knaus, Evanston, Illinois. A recent paper in the Journal of Reproductive Medicine addressed the issue of Papanicolaou smear accuracy.[1] The training level completed and the number of years of clinical practice of the person performing the smear did not equate with greater accuracy in obtaining endocervical cells. The authors concluded that obtaining an adequate Papanicolaou smear was as much an art as a science.

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Dr. McCord (Closing). I begin by addressing Dr. Knaus's questions. Whether the presence of endocervical cells will improve the detection of dysplasia is unclear. However, most studies have shown that the presence of endocervical cells on the smear is associated with improved smear quality. This, then, appears to be correlated with adequate sampling of the entire transformation zone.

Because we did not detect any significant differences in the sampling methods for the nonpregnant patient, including improved detection of dysplasia, the cotton swab is the most cost-effective method of screening for the nonpregnant patient. In the nonpregnant patient there was no significant difference in the presence of endocervical cells with any of the four methods.

Dr. Schink asked several questions. To begin, let me explain why we chose to include subjective criteria in our study. Our laboratory records the reason a Papanicolaou smear is considered inadequate. Frequently a Papanicolaou smear is unsatisfactory for reasons other than scant cellularity. Some of the reasons may be sampling-method dependent: excessive blood and air-drying effect. Other reasons, such as excessive inflammation, are not method dependent. A sampling method that collects endocervical cells yet results in poor quality smears because of cell clumping, bleeding,

or air drying should not be used. Unfortunately these criteria are subjective and vary between laboratories and individuals. The very nature of cytology and diagnosis of dysplasia is subjective.

I defer to Dr. Schink his comments on the statistical adequacy of our patient population. Our statistical data was performed by a biostatistician who assured us before the initiation of our study that our sample size was adequate.

It was noted that our conclusion for nonpregnant patients differed from other studies. Most studies evaluating sampling methods have addressed the recovery of endocervical cells as the sole criterion for recommending a method. Endocervical cell recovery is extremely variable. For instance, Murata et al. recommended use of the Cytobrush over the cotton swab as the endocervical detection rate improved from 62% to 83%. We had a detection rate of 90% for nonpregnant patients with the cotton swab. Again, this demonstrates the importance of understanding the criteria used in your laboratory.

We believe that inadequate smears, for whatever reason, should be repeated.

Both the Cytobrush and the Bayne Pap Brush have qualifiers on their packages cautioning about use in pregnancy. Before beginning our study, I spoke with representatives from both companies. Both have unpublished data documenting the safety of these devices in pregnancy. The disqualifiers remain on the packages because the data is unpublished. We did not have any known complications from any sampling methods. Patients in their third trimester of pregnancy tended to have cervical bleeding when the Papanicolaou smear was obtained with the Bayne Pap Brush. Two patients were kept in the clinical area for 1 to 2 hours for observation. Interestingly, the incidence of excess blood on smears was not increased for patients in the third trimester of pregnancy.

We used wooden spatulas with the cotton swab and Cytobrush. The use of the plastic Ayre spatula might have improved our smear quality further.

Interlaboratory variation in antiphospholipid antibody testing

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OBJECTIVE: Because of the widespread use of antiphospholipid antibody testing in the evaluation of patients with recurrent pregnancy loss, we evaluated the consistency of results among laboratories testing for anticardiolipin antibody and the lupus anticoagulant.

STUDY DESIGN: A questionnaire regarding methods used and samples of blood from 20 patients were sent to five university-based and five commercial facilities for antiphospholipid antibody testing.

RESULTS: The responses of the participating laboratories to the questionnaires revealed significant differences in methods, standardization, and units of reporting. For anticardiolipin antibody, the number of specimens found to be positive for any isotype (immunoglobulin G, M, or A) varied considerably among laboratories, with a range of 5 to 13. All laboratories were in agreement (i.e., at least one isotype was present or all were absent) for only 5 of 20 specimens (25%). In contrast, lupus anticoagulant results were more reproducible, although one facility reported results markedly discordant from the other four laboratories.

CONCLUSION: These observations suggest that significant interlaboratory variation exists in antiphospholipid antibody, and particularly anticardiolipin antibody, testing and might lead to unnecessary therapeutic interventions. (Am J Obstet Gynecol 1992;166:1780-7.)

Key words: Antiphospholipid antibodies, lupus anticoagulant, anticardiolipin antibody, recurrent pregnancy loss

Antiphospholipid antibodies are circulating autoantibodies directed against negatively charged phospholipid components of cell membranes. Two particular antiphospholipid antibodies, anticardiolipin antibody and the lupus anticoagulant, have recently been implicated as possible causes of recurrent adverse obstetric outcomes. Second-trimester fetal death, early severe preeclampsia, fetal growth retardation, and maternal thrombosis have all been associated with the presence of lupus anticoagulant and anticardiolipin antibody, and these complications have been suggested as indications for antiphospholipid antibody testing. Although testing for lupus anticoagulant and anticardiolipin antibody is currently being performed by many laboratories in the United States, the College of American Pathologists does not perform proficiency testing or standardization for either assay. A method of standardized testing was proposed by the Kingston Antiphospholipid Antibody Study group at the anticardiolipin standardization workshop,[1] but these recommendations have not been accepted by all laboratories.

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Therapy for prevention of poor pregnancy outcome in patients who have positive test results for antiphospholipid antibodies has been largely empiric and has included glucocorticoids,[2,3] aspirin,[2,3] heparin,[4] plasma exchange,[5] and intravenous immune globulin.[6] If significant variation exists among laboratories, it would likely result in either large numbers of patients receiving unnecessary therapies or repeat pregnancy losses in untreated patients who have false-negative results. On numerous occasions we have encountered discordant results among referral patients who have had testing in two different laboratories. These clinical observations prompted us to conduct this investigation to determine whether significant variation in either the method used or the results exists among regional and national laboratories testing for antiphospholipid antibodies.

Methods

Ten laboratories were chosen for participation in this investigation, five that were associated with university departments and five that were independent commercial laboratories. Questionnaires were sent to participants asking for (1) number of samples tested annually, (2) instructions for sample preparation and shipping, (3) description of testing used, (4) methods of standardization, and (5) units for reporting results. All laboratories approached agreed to participate with the understanding that participating centers would not be identified by name. All 10 facilities performed anticar-

Table I. Selected characteristics of participating anticardiolipin antibody laboratories

Laboratory	*No. of samples tested annually*	*Isotype tested*	*Units reported*
University			
A	6,000	IgG, IgM	GPL, MPL
B	1,532	IgG, IgM	GPL, MPL
C	40	IgG, IgM, IgA	High, moderate, low
D	18,000	IgG, IgM	Titer
E	*	IgG, IgM	GPL, MPL
Commercial			
F	3,560	IgG, IgM	Titer
G	68,400	IgG, IgM, IgA	GPL, MPL, APL
H	2,400	IgG, IgM, IgA	GPL, MPL, APL
I	400	IgG, IgM, IgA	GPL, MPL, APL
J	1,250	IgG, IgM	GPL, MPL

GPL, MPL, and APL are units corresponding to the binding activity of 1 μg/ml of purified anticardiolipin antibody (IgG, IgM, and IgA, respectively).

*New assay.

diolipin antibody testing, but only five performed assays for lupus anticoagulant.

Phlebotomy was performed on 20 nonpregnant, consenting subjects who were not receiving steroids, aspirin, or anticoagulant therapy at the time. Plasma and serum were extracted in a routine manner (with and without sodium citrate, respectively, then centrifuged at 3000*g* for 30 minutes) and stored at −20° C. Twelve of the 20 samples were from patients who previously had positive test results for at least one antiphospholipid antibody, the initial assay being performed in response to a prior medical or obstetric complication. Two additional patients with normal obstetric histories and no prior testing were also included. The remaining six control specimens were obtained from the antiphospholipid antibody laboratory at the University of Utah and were known to be anticardiolipin antibody positive ($n = 3$), anticardiolipin antibody negative ($n = 2$), or borderline anticardiolipin antibody positive ($n = 1$). After all specimens were obtained, samples were thawed, separated into aliquots, coded, and distributed to all participating centers without patient identification or clinical history. Institutional review board approval was obtained before performance of this study.

Results

All laboratories performed assays for immunoglobulin (Ig) G and IgM anticardiolipin antibodies with an enzyme-linked immunosorbent assay; four of 10 were tested for IgA anticardiolipin antibody as well (Table I). Five of the laboratories requested frozen specimens sent on dry ice, with the others requesting overnight

Table II. Selected characteristics of participating lupus anticoagulant laboratories

Laboratory	*No. of samples tested annually*	*Method used*	*Units reported*
University			
A	2400	Partial thromboplastin time	Seconds
C	40	Dilute Russell viper venom test	Seconds
E	300	Dilute Russell viper venom test	Seconds
Commercial			
F	3500	Partial thromboplastin time	Positive or negative
I	200	Kaolin clotting time	Positive or negative

delivery of fresh sera. Seven laboratories claimed to have standardized their assays with samples derived from the Kingston Antiphospholipid Antibody Study program.[1] Seven of the 10 laboratories reported results in terms of GPL, MPL, and APL units, which correspond to the binding activity of 1 μg/ml of purified anticardiolipin antibody for IgG, IgM, and IgA, respectively[1]; six of these seven also provided qualitative interpretation of positive values as low, moderate, or high. Two laboratories reported positive results as titers without qualitative interpretation, and one reported positive results only as low, moderate, or high.

The questionnaire responses with regard to lupus anticoagulant testing methods are described in Table II. Two laboratories measured partial thromboplastin times, whereas the other three laboratories used viper venom tests or the kaolin clotting time as their primary assay. Three participants required that samples be received frozen, and one specifically requested that the samples be filtered to remove platelets before freezing. Three of the five laboratories were standardized against samples from a different laboratory. Although all tests were bioassays of in vitro clotting function, two laboratories characterized results only as positive or negative and gave no indication of the degree of abnormality.

The clinical histories of the patients from whom samples were obtained are summarized in Table III, along with subsequent treatment and pregnancy outcomes.

The results of serum anticardiolipin antibody testing are now displayed in Table IV. If the presence of any isotype (IgG, IgM, or IgA) at any level is considered a positive result, the number of specimens found to be positive by each laboratory ranged from 5 to 13. For only 5 of 20 specimens (25%) were all laboratories in agreement (i.e., at least one isotype was present or all were absent). For another six patients, all laboratories with one exception were in agreement. For the remaining nine samples tested (45%), there was marked

Table III. Clinical histories of participating patients

Patient no.	*Medical history*	*Obstetric history before initial testing*	*Subsequent pregnancy*	*Antiphospholipid therapy*
1	Mixed connective tissue disease	Gravida 2, para 0-2-0-2; 25 wk intrauterine fetal death, 31 wk twins	35 wk GA	Aspirin, prednisone
2	Scleroderma	Gravida 1	Term AGA	Aspirin, prednisone
3	Systemic lupus erythematosus, chronic hypertension	Gravida 7, para 0-1-5-1; 4 first-trimester losses, 17 wk loss, 35 wk IUGR	Pregnancy-induced hypertension, placentae abruptio at 32 wks	Aspirin, prednisone
4	Prior cerebrovascular accident	Gravida 3, para 1-1-0-1; 29 wk intrauterine fetal death, term AGA	Term AGA	None
5	None	Gravida 3, para 0-0-2-0; 2 24 wk intrauterine fetal deaths	26 wk IUGR, neonatal death	None
6	None	Gravida 2, para 0-1-0-1; 31 wk, severe pregnancy-induced hypertension, IUGR	Term AGA	Aspirin, prednisone
7	None	Gravida 3, para 0-1-1-0; 28 wk, IUGR, neonatal death	Term AGA	Aspirin
8	None	Gravida 5, para 1-0-3-1; 3 first-trimester losses	Term AGA	None
9	None	Gravida 5, para 1-0-3-1; 3 first-trimester losses	Term AGA	None
10	None	Gravida 4, para 1-0-3-1; 3 first-trimester losses	—	—
11	None	Gravida 1, para 0-1-0-1; severe pregnancy-induced hypertension at 29 wk	—	—
12	Positive antinuclear antibody, positive Venereal Disease Research Laboratory test	Gravida 5, para 3-1-0-4; 34 wk AGA	Term AGA	None
13	None	Gravida 1	—	—
14	None	Gravida 1	—	—
15	Known high positive			
16	Known high positive	—	—	—
17	Known high positive	—	—	—
18	Known negative	—	—	—
19	Known negative	—	—	—
20	Known borderline positive	—	—	—

IUGR, Intrauterine growth retardation; *AGA*, appropriate for gestational age.

variation among laboratories regarding the presence of anticardiolipin antibody.

Plasma was available for lupus anticoagulant testing from only 10 patients (Table V). Although fewer laboratories participated, there was less interlaboratory variation with anticardiolipin antibody testing. The results from one laboratory (I), however, were significantly different from the others, with lupus anticoagulant found in 6 of the 10 specimens tested. Among the other four laboratories there was agreement on all specimens but one, for which one laboratory reported the specimen as negative and the other three laboratories detected the presence of lupus anticoagulant.

Comment

During the last decade an increased awareness of potential complications associated with antiphospholipid antibodies has prompted a dramatic increase in testing for anticardiolipin antibody and lupus anticoagulant. Both hospital-associated and commercial laboratories have expanded antiphospholipid antibody testing programs in response to this clinical demand. However, not until 1986 was the possibility of significant interlaboratory variation in antiphospholipid antibody testing formally considered. For anticardiolipin antibody an attempt at standardization of testing was initiated with the first Kingston Antiphospholipid Antibody Study meeting, which resulted in a multicenter comparison of samples.[1] In this study purified anticardiolipin antibody samples from patients with the "antiphospholipid antibody syndrome" were sent to 30 participating university laboratories with instructions on how to prepare the samples for testing. Although there was general agreement in results from samples with high levels of anticardiolipin antibody, considerable variation was found for those sera with lower values. The most recent Kingston Antiphospholipid Antibody Study standardization workshop demonstrated

Table IV. Anticardiolipin antibody results

Patient no.	Laboratory A	B	C	D	E	F	G	H	I	J	Anticardiolipin antibody positive
1	LP/MP	NA	−/LP/−	+/+	−/−	+/+	LP/−/−	−/−/−	LP/LP/MP	LP/−	7
2	LP/−	HP/−	−/−/−	+/−	+/−	+/−	LP/−/−	−/−/−	−/−/−	−/−	6
3	MP/HP	MP/HP	−/LP/LP	+/+	−/+	+/+	MP/MP/MP	MP/MP/−	MP/MP/LP	MP/HP	10
4	−/−	−/−	−/−/−	−/−	−/−	−/−	−/−/MP	−/−/−	−/−/−	−/−	1
5	−/−	−/−	−/−/−	−/NA	−/−	−/−	−/−/−	−/−/−	LP/−/−	−/−	1
6	−/−	LP/−	−/−/−	NA	−/−	+/−	LP/−/−	−/−/−	−/−/−	−/−	3
7	−/−	MP/−	−/−/−	−/−	−/−	−/+	LP/−/−	−/−/−	−/−/−	−/−	3
8	−/−	MP/−	−/−/−	−/−	−/−	−/−	−/−/−	−/−/−	−/−/−	−/−	1
9	−/−	NA	−/−/−	−/−	−/−	−/+	−/−/−	−/−/−	−/−/−	−/−	1
10	−/−	−/−	−/−/−	−/+	−/−	−/−	−/−/−	−/−/−	−/−/LP	−/−	2
11	LP/−	LP/−	−/−/−	−/−	−/−	+/+	LP/−/−	−/−/−	−/−/−	−/−	4
12	−/−	LP/−	−/−/LP	−/−	−/−	+/−	LP/−/−	−/−/−	LP/−/−	−/−	5
13	−/−	NA	−/−/−	−/−	−/−	−/−	−/−/−	−/−/−	−/−/−	−/−	0
14	−/−	MP/−	−/−/−	−/−	−/−	−/+	−/−/−	−/−/−	−/−/−	−/−	2
15	HP/LP	HP/HP	−/−/−	+/NA	−/+	+/+	MP/−/−	LP/−/−	MP/−/MP	MP/LP	9
16	−/HP	HP/HP	HP/MP/LP	+/NA	−/+	+/+	HP/MP/HP	HP/HP/LP	HP/HP/LP	HP/HP	10
17	MP/HP	NA	LP/HP/−	NA	−/+	+/+	MP/MP/MP	MP/MP/−	HP/HP/LP	MP/MP	8
18	−/−	LP/−	−/−/−	−/−	−/−	−/−	−/−/−	−/−/−	−/−/−	−/−	1
19	−/−	−/−	−/−/−	−/NA	−/−	−/−	−/−/−	−/−/−	NA	−/−	0
20	MP/−	LP/−	−/−/−	+/−	−/−	+/−	MP/−/−	MP/−/−	−/−/−	LP/−	7
TOTAL POSITIVE	8	12	5	7	5	13	12	5	8	6	

Results are given as IgG/IgM or IgG/IgM/IgA. Results are listed at positive (+) or negative (−) unless qualitative interpretation was provided. *HP, MP, LP,* High-, moderate-, and low-positive results; *NA,* result not given.

continued interlaboratory variation among participants for low- and medium-positive values of anticardiolipin antibody.[7] No similar laboratory comparisons have been undertaken for lupus anticoagulant.

Coulam et al.[8] tested samples from 20 women with recurrent spontaneous abortion in four laboratories and concluded that the results of anticardiolipin antibody testing were not reproducible. Only two of eight specimens found to have elevations of anticardiolipin antibody in one laboratory were confirmed as positive in a second facility. These authors questioned the usefulness of a single anticardiolipin antibody test for assessing women with reproductive loss.

Our data also suggest that significant interlaboratory variation exists in anticardiolipin antibody testing. Marked variation in test results among laboratories was found for almost one half of the samples studied. The variation in positivity was not simply a result of differences in determining a mutually agreeable cutoff value (i.e., sensitivity); these differences persisted even when low values were disregarded. Even when there was agreement on the presence of anticardiolipin antibody, there were obvious qualitative differences. We did not assess intralaboratory variation, but prior studies suggest that it may also be a source of substantial variation.[1] These observations suggest that when clinical decisions are based on a single test result detection of anticardiolipin antibody and recommendations for therapy will be highly dependent on which laboratory is used.

Table V. Lupus anticoagulant results

Patient no.	Laboratory A	C	E	F	I
1	−	−	−	−	+
2	−	−	−	−	−
3	+	+	+	−	+
4	−	−	−	−	−
6	−	−	−	−	−
7	−	−	−	−	+
8	−	−	−	−	−
9	−	−	−	−	+
11	−	−	−	−	+
12	−	−	−	−	+

For lupus anticoagulant, interlaboratory comparison demonstrated less variation, a finding consistent with a prior investigation involving hospital laboratories in the United Kingdom.[9] In that study >90% of the laboratories correctly identified negative and strongly positive lupus anticoagulant samples, although sample preparation and assay method used were not standardized.

Clinical consequences could result from a false-positive antiphospholipid antibody result. First, if a positive

result were used to explain a patient's pregnancy losses, other causes deserving further investigation might go unexplored. Second, medical therapy with agents such as prednisone, aspirin, or heparin, if prescribed, would expose the patient to potentially serious risks without therapeutic benefit. The efficacy of these agents has never been tested in a placebo-controlled study in patients with confirmed high levels of anticardiolipin antibody; their therapeutic value is surely in question if the presence of anticardiolipin antibody is uncertain.

The lack of an identified reference laboratory prevents differentiation of discrepant results into false-positive or false-negative ones. We do note, however, that four of our patients with testing discrepancies had normal pregnancy outcomes without therapy for anticardiolipin antibody. Although it is possible that some patients with false-negative or discrepant test results might benefit from empiric therapy, it is more likely that many women are being unnecessarily exposed to the risks of medical treatment.

On the basis of these observations, we advocate the confirmation of a positive result for anticardiolipin antibody in another laboratory before recommendations for therapy are given. This practice would most likely reduce the number of patients being treated, with only a small chance of withholding therapy from a patient who has positive test results in a majority of laboratories. Because many centers have altered and improved their methods over the past 5 years, we also recommend that testing be repeated for patients who have positive results and have been treated for anticardiolipin antibody in prior pregnancies. In contrast, for lupus anticoagulant repeat testing would be less likely to identify any inconsistencies in results.

We thank D. Ware Branch, MD, for providing the control samples for this project.

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Discussion

Dr. Edmond Confino, Chicago, Illinois. The association between elevated antiphospholipid antibody, abnormal lupus anticoagulant, and repeated pregnancy loss has been repeatedly documented.[1] However, the best treatment modality remains controversial, and our inability to determine that best treatment modality for patients with this syndrome reflects not only lack of adequately documented prospective, randomized clinical trials but also an inconsistency in the laboratory determination of abnormal levels of anticardiolipin antibody and lupus anticoagulant.[2] This observation prompted the organization of the second international anticardiolipin standardization workshop,[3] which unfortunately was not followed by all clinically involved laboratories worldwide and nationwide.

The authors used anticardiolipin antibody and lupus anticoagulant samples obtained from a single center. These blood samples defined the strongly positive, negative, and borderline values. Although this approach is appropriate, false-positive values may occur in any reference laboratory as well. It is probable that only adaptation of the Kingston Antiphospholipid Antibody Study recommendations will resolve methodologic and reference concerns in those assays.

It is surprising that the interlaboratory differences in the determination of lupus anticoagulant were lower then the differences measured for anticardiolipin antibody. The lupus anticoagulant was measured by different methods such as activated partial thromboplastin time, viper venom testing, and kaolin clotting time. Although it is intuitively expected that differences may be larger with lupus anticoagulant, other authors also reported smaller interlaboratory differences in the designation of strongly positive cases.[4] The search for a reference laboratory determination for lupus anticoagulant is still continuing, and it seems that these bioassays that indirectly measure the ill-defined lupus anticoagulant require further definition and development.[5]

The argument of the authors that positive anticardiolipin antibody and lupus anticoagulant values may potentially interfere with complete evaluation of patients with repeated pregnancy loss is questionable, because it assumes inappropriate medical decision-making. In a similar fashion the argument that false-positive lupus anticoagulant and anticardiolipin antibody determinations will expose patients to unnecessary risky treatments is also debatable, because steroid, aspirin, and heparin treatments are considered experimental and they should be approached and administered as such.

This study further emphasizes the already well-known message that clinical application of lupus anticoagulant and anticardiolipin antibody determinations, and the subsequent treatment of affected patients,

should be regarded as experimental only. Until adequate referencing and method standardization are achieved, physicians are advised to follow the recommendation of the authors to consider positivity only when corroborated by several laboratories and use the information from such testing with cautious critical approach.

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Dr. Paul G. Tomich, Maywood, Illinois. Dr. Peaceman and associates have raised the question, "Is there interlaboratory variation in antiphospholipid antibody testing?" Blood specimen results were recorded and the data analyzed for 10 laboratories (five university based and five commercial) and 20 patients (12 of whom previously had positive test results to at least one antiphospholipid antibody, two with normal obstetric histories without prior testing, and six University of Utah control patients).

The study results demonstrate the following:

1. There is significant interlaboratory variation in both anticardiolipin antibody and lupus anticoagulant testing.
2. Lupus anticoagulant results are more reproducible than are anticardiolipin antibody test results.
3. There was excellent correlation with interlaboratory data and the University of Utah control patients.
4. There was minimal interlaboratory variation in those patients with obstetric or medical histories associated with the antiphospholipid antibody syndrome.

These results are not surprising. Since 1983, when Harris[1] introduced the solid phase of the anticardiolipin test, one of the major clinical dilemmas for the practicing obstetrician-gynecologist has been making the laboratory diagnosis of the antiphospholipid antibody syndrome. To that end Harris has made standard positive sera available to several laboratories nationally so that results may be presented in a repeatable quantitative fashion that is referable to an international experience. Unfortunately, as this study demonstrates, some laboratories have not yet standardized their assays with others. Hence physicians are faced with the clinical dilemma of whether to treat even if the patient does not have a history for the antiphospholipid antibody syndrome. This lack of standardization is also a major problem in interpreting clinical data on the antiphospholipid antibody syndrome.

In obstetrics the antiphospholipid antibody syndrome has been associated with a variety of clinical situations: recurrent pregnancy losses, early-onset preeclampsia, stillbirths, intrauterine growth retardation, and other rare obstetric complications.[2-5]

Approximately 2% of low-risk obstetric patients can be identified as having positive results, with poor pregnancy outcomes.[6] Low-positive values are probably not of significance and transient-positive results do occur. IgG antibodies are of more significance than IgM antibody detection.

The implications of substantial variation in laboratory results is obvious. If the result is false positive, overtreatment will occur. If the result is false negative, then undertreatment will occur.

What does this mean to the practicing obstetrician-gynecologist? Dr. Peaceman has suggested that a second anticardiolipin antibody test should be performed. I would suggest that the clinician should use a laboratory where an appropriate immunoassay procedure and report results are presented in a semiquantitative manner with reference to the sera made available by Harris.

I would like to ask Dr. Peaceman three questions:

1. Do you have any information concerning the variation in cost of such testing at each of the laboratories used in your study?
2. Do you have an explanation as to why three of the 10 laboratories have not been standardized by the Kingston Antiphospholipid Antibody Study?
3. In your own referral practice have you developed confidence in your own laboratory, such that you would consider action on those results rather than recommending that another sample be sent out?

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Dr. Alan E. Beer, Chicago, Illinois. I would like to ask Dr. Peaceman if he has made a clinical correlation with the laboratory results and if he has also addressed the intraassay and interassay coefficients of variability of the various reporting laboratories. If these are not established, the results that were presented could be meaningless because of lack of this standardization.

Dr. Robert J. Carpenter, Houston, Texas. There's a significant difference in the number of patients stud-

ied in different laboratories, all the way from a low of 40 to a high of 68,400. If one excludes the five lowest laboratories, which all have studies of <2000, is there any closer agreement among the five highest? And for those who have not read it, there's an article in *The New England Journal of Medicine*[1] that puts this whole issue into even greater turmoil.

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DR. WASHINGTON C. HILL, Nashville, Tennessee. Your recommendation is that if I send a test off to a laboratory and I get back a positive result, then I need to send it off to another laboratory to see what the laboratory tells me. I'm a little confused with that because then I won't know which test I'm supposed to believe. I think Dr. Tomich's points are well taken. Perhaps, instead of sending the test off to another laboratory, we need to get to know the laboratory where we're having the first test done.

DR. MARVIN L. HAGE, Durham, North Carolina. I would ask the authors if they could present their data taking into account chance agreement. This is an important consideration whether between laboratories or within laboratories. The statistic frequently cited is κ, a statistic that varies between +1 and −1. Second, were any tests of significance used?

DR. JOHN L. AUSTIN, Memphis, Tennessee. I understood the method used in that the specimens were collected, frozen, and then simultaneously thawed and separated into aliquots. What was the variation from collection to the time of testing? I assume that with 10 laboratories there was a transit variable. How stable were these substances in transit, and what were the conditions of transit? Are they similar from laboratory to laboratory?

DR. PEACEMAN (Closing). First, Dr. Confino talked about Dr. Harris's previous studies showing there was a good correlation among different laboratories. What Dr. Harris did report was that when he took samples that were known to be highly positive for anticardiolipin antibody and sent them to academic centers around the world with instructions as to how to prepare the samples, he was able to get a good correlation. In each of his two articles on the subject, however, he cautioned that with low or moderate levels significant variation was seen. We deal mostly with these low or moderate values on a day-to-day basis. So, although he described more correlation than we found, he was also disturbed by the fact that there was less agreement for the samples with low levels of anticardiolipin antibody.

The other issue to remember is that he submitted the samples the way he wanted them run. Our study was very different. We were trying to evaluate current laboratory practice. In other words we submitted the samples in the way each laboratory asked for them and instructed the laboratories to run the samples according to their usual protocols, because that's what happens in everyday testing. We think that as such it better addresses the question as to whether there will be interlaboratory variation among patient care samples.

I agree that part of the discrepancy may be due to differences in calibration among the 10 different laboratories; but once again, this doesn't matter. In terms of our study design, our aim was not to try to correct the laboratories. The goal was to determine whether a sample sent to different laboratories will get different results. To make a decision on therapy on the basis of a single result does not recognize the fact that the issue of positivity may still be in doubt. We think the first step in correcting the problem is indeed recognizing that a problem with testing exists; and for most practicing physicians there is really no recognition that interlaboratory variation exists.

We are glad that Dr. Confino shares our bias against treatment in many of these patients. We are not necessarily advocating treatment if two laboratories are in agreement. Rather, our recommendation is limited to suggesting that if significant interlaboratory variation is identified treatment should not be prescribed until the issue can be clarified.

In answer to Dr. Tomich's question, no, we didn't collect any cost data, although that would be interesting to assess. There are a number of laboratories that are not testing for only one or two antiphospholipid antibodies but running panels testing for more than 20 different antibodies; these tests can be extremely expensive.

We don't know why the laboratories all haven't standardized their protocols. Perhaps the laboratories themselves don't recognize the possibility of interlaboratory variation. Indeed, when some of the directors were contacted, they seemed quite shocked by our results.

Dr. Tomich asked if we trusted the results from our own laboratory. In our hospital samples for anticardiolipin testing are sent out to a commercial laboratory—one of the commercial laboratories tested here. The answer to his question is, no, I don't trust that laboratory entirely. I use the laboratory at the University of Utah for confirming positive results. The last six specimens in this study were obtained from that laboratory, and there was significant agreement among all of the different laboratories for those specimens.

Dr. Beer asked about clinical correlation. With patients who have had a positive test result, we send a second sample off as I described. For those samples that come back with variation in testing results, we suggest that treatment is not necessary. We see such variation in approximately 90% of the patients that are referred to us for testing, and indeed most do well in subsequent pregnancies without therapy. Our clinical suspicion is that most intralaboratory variation represents false-positive rather than false-negative results. Although this is our clinical suspicion, we have not performed a prospective trial to support this contention with strong data.

In terms of intraassay variation, prior studies have shown this to be significant as well. We did not assess this particular issue, but we feel that the interlaboratory variation is significant enough that by itself it calls into question whether treatment is necessary.

Dr. Carpenter asked about excluding the laboratories doing the fewest number of tests. That doesn't solve the problem; unfortunately, it makes it worse. The laboratories with the highest volumes were the ones we thought had the most discrepant results, and that's what we found extremely worrisome. They were the ones doing most of the testing for which recommendations for therapy are being made.

Dr. Hill asked about repeat testing and why two suspicious results might be better than one. Because our clinical experience suggests that discrepant results most likely represent false-positive values, we believe repeat testing will allow many women to avoid the hazards of treatment. Even among the seven laboratories that had claimed standardization with the Kingston Antiphospholipid Antibody Study study, there was significant variation in testing results. Standardizing against Dr. Harris specimens therefore does not guarantee uniformity.

Statistical analysis of this study was not presented. We could not find an appropriate statistical method to evaluate the data for significance of variation. The degree of agreement that was seen is greater than would occur by random chance.

Reproductive outcome in women with recurrent spontaneous abortions of alloimmune and autoimmune causes: Preconception versus postconception treatment

Joanne Y.H. Kwak, MD, Alice Gilman-Sachs, PhD, Kenneth D. Beaman, PhD, and Alan E. Beer, MD

North Chicago, Illinois

OBJECTIVES: The null hypothesis is that treatment of women with recurrent spontaneous abortions with anticoagulation and immunosuppression will not increase the reproductive outcome if it is started preconceptionally.

STUDY DESIGN: Ninety-four women with recurrent spontaneous abortion with autoimmune abnormalities comprised the study group. Group I began autoimmune therapy 48 hours after ovulation: heparin 5000 U twice daily, aspirin 80 mg daily, and prednisone 5 mg twice daily, with an increase to 10 mg twice daily when pregnant. Group II started the same medication after a positive pregnancy test. Group III received no medication. Controls were 19 women with no autoimmune abnormalities. The frequency of reproductive outcome was subject to multiple comparison by the Duncan test.

RESULTS: The percentages of live-born children in groups I, II, and III were 74%, 44%, and 11%, respectively.

CONCLUSIONS: Preconception diagnostic work-up and treatment of autoimmune abnormalities in women with histories of recurrent spontaneous abortion is advocated. (Am J Obstet Gynecol 1992;166:1787-98.)

Key words: Abortion, autoimmune, treatment, preconception

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6/6/37127

We have previously evaluated both the maternal antipaternal alloimmune and autoimmune status of women with unexplained recurrent spontaneous abortions, women with normal pregnancies, and women who terminate their pregnancies electively early in gestation and have found major differences in the alloimmune and autoimmune status in women with unexplained recurrent spontaneous abortions.[1, 2] In addi-

tion, it has been reported that autoimmunity to certain negatively charged phospholipid molecules can activate phospholipid-dependent coagulation processes in the mother and interfere with phospholipid adhesion molecules thought necessary for cytotrophoblast to syncytiotrophoblast development.[3-5] Women in this category experience a higher incidence of recurrent spontaneous abortion and pregnancy complications. The role that the presence or absence of maternal alloimmunity to paternal antigens and autoimmunity to phospholipid antibodies play in the success or failure of pregnancy is far less well established and still controversial.[6] To address these alloimmune and autoimmune questions we tested women with recurrent spontaneous abortions of unknown cause for alloimmune recognition of paternal antigens and autoimmune responses before their subsequent pregnancy under study. Two hundred seventy women with recurrent spontaneous abortions lacked evidence of prior maternal antipaternal immune recognition and were immunized with paternal or donor lymphocytes. Once adequate levels of antibody were induced, all couples entered a cycle of planned conception. Before attempting pregnancy, 94 consecutive women were delegated to a treatment subgroup. Nineteen women had no autoimmune abnormalities and received no further treatment before or during pregnancy. The remainder of the women with autoimmune abnormalities were prospectively designated to three groups that received (I) preconception treatment starting at the onset of the cycle of conception, (II) postconception treatment with the first positive pregnancy test, and (III) no autoimmune treatment.

We reasoned that this prospective study could provide data that might give insight and answers to a poorly studied and controversial area regarding appropriate therapy for women with recurrent spontaneous abortions. We also reasoned that if antiphospholipid antibodies are capable of altering or unsticking important phospholipid adhesion molecules necessary for placental organ development, a preconception autoimmune treatment may result in a better reproductive outcome than a treatment started after pregnancy is clinically diagnosed or no treatment at all.

Material and methods

Patients. Women with recurrent spontaneous abortions were physician-referred to the Reproductive Immunology Clinic, University of Health Sciences/Chicago Medical School. All were completely evaluated clinically before the immunologic work-up to rule out genetic, structural, endocrine, and infectious causes. Women who had clinically explainable cause(s) for their previous pregnancy losses were excluded. Women who were pregnant at the initial work-up and who had histories of molar pregnancies, therapeutic abortions, and miscarriage after 24-week gestations were excluded from this study. Women with adequate blocking antibody on initial evaluation were also excluded. From these patients 270 women with three or more recurrent spontaneous abortions who showed lack of alloimmune responses were immunized with lymphocytes and comprised the study group. One hundred eleven women in the study group who demonstrated positive antiphospholipid antibodies comprised the treatment group and were prospectively designated to three treatment subgroups before index pregnancy: (I) preconception treatment starting at the onset of the cycle of conception, (II) postconception treatment with the first positive pregnancy test, and (III) no autoimmune treatment. Ninety-four women became pregnant and are reported in this study.

Alloimmune evaluation. Human leukocyte antigen (HLA) tissue typing, mixed lymphocyte culture reactivity, mixed lymphocyte blocking activity, maternal antipaternal antibody assessment by crossmatch with microcytotoxicity, and flow cytometry were conducted as previously reported.[7, 8] The absence of maternal antipaternal T- and B-cell antibodies before immunization (<30% anti-T- and anti-B-cell antibodies) coupled with absent blocking activity (<50%) in mixed lymphocyte culture constituted a lack of alloimmune recognition. Each women in this category was immunized with paternal or nonpaternal donor lymphocytes as previously reported.[9] Postimmunization evaluation documented an adequate response in all.

Autoimmune evaluation. Antibodies to phospholipid and antinuclear antibody were evaluated before immunization and before pregnancy. Once pregnancy was established, these were repeated every 2 weeks. The dynamics of alloantibody and autoantibody levels during pregnancy are not discussed in this article and are the subject of another report.

Antiphospholipid antibody. Antiphospholipid antibodies were tested by enzyme-linked immunosorbent assay.[10, 11] Polystyrene microtiter plates were coated with 30 μl of cardiolipin (45 μg/ml in ethanol) or one of five other phospholipids (50 μg/ml in methanol), that is, phosphatidylserine, phosphatidylinositol, phosphatidylethanolamine, phosphatidyl glycerol, or phosphatidic acid. The plates were allowed to dry overnight at 4° C. After drying, the plates were washed once with phosphate buffered saline, blocked for 2 hours at room temperature with phosphate buffered saline solution containing 10% fetal calf serum, then washed once more. Serum from patients and standards diluted 1:50 in phosphate buffered saline solution containing 10% fetal calf serum were added to the wells. The plates were incubated for 2 hours at room temperature, then washed three times with phosphate buffered saline so-

lution. Fifty μl of indirect antibody (alkaline phosphatase conjugated antihuman immunoglobulin [Ig] G, IgM or IgA) at saturating concentrations were added to the appropriated wells and were incubated for 1 hour. After the incubation the plates were washed three times in phosphate buffered saline solution and 50 μl of *p*-nitrophenyl phosphate (1 mg/ml) were added to each well. After 30 minutes the reaction was stopped by the addition of 50 μl of 3 mol sodium hydroxide to each well. The optical density of each well was determined with a plate reader (Dynatech, Chantilly, Va.) at 405 nm. The Immunosoft program (Dynatech) was used to average duplicate wells and subtract the nonspecific background for each antibody specificity for each class of duplicate wells. Each plate was run with a positive and negative control as standards. Background optical density was determined by running the assay with patient serum diluted 1:50 in 10% fetal calf serum in phosphate buffered saline solution in wells after the same microtiter plates coated with ethanol or methanol but no phospholipids.[12] Cut-off values were determined with sera from 60 nonpregnant healthy women between the ages of 20 and 45. An absorbance ≥3 SD above the mean for that phospholipid, and antibody isotype was considered positive.

Antinuclear antibody. Antinuclear antibodies were measured by indirect immunofluorescence with human laryngeal tumor cells as substrate. Sera were screened for antinuclear antibodies activity at a dilution of 1:40. Those displaying 1+ or greater fluorescence at dilution of 1:40 were considered positive. Positive sera were then retested at dilutions of 1:80, 1:160, and higher when necessary.

Controls. Ninety normal women with documented uncomplicated pregnancies and no history of spontaneous or therapeutic abortions were tested as normal multiparous controls. All control subjects were interviewed, during which time personal and family histories were ascertained. None had an autoimmune disorder, and all experienced the live birth of a healthy baby. Age and gravidity of the control group were matched with those of women with recurrent spontaneous abortions in the study group.

Nineteen women from the lymphocyte-immunized group had no autoimmune abnormalities before and during their pregnancies. They were used as nonautoimmune controls for those immunized women with autoimmune abnormalities.

Medication and obstetric management. Women in group I received preconception treatment with low-dose aspirin, heparin, and prednisone. Patients began autoimmune therapy as follows: aspirin 80 mg daily, prednisone 5 mg twice daily starting day 1 of conception cycle, and heparin 5000 U twice daily administered subcutaneously by self-administration starting 48 hours after ovulation. Prednisone was increased to 10 mg orally twice daily, with a positive β-human chorionic gonadotropin test conducted on days 26 through 28 of the cycle. Group II women received postconception treatment at the time of a positive β-human chorionic gonadotropin test conducted at the end of the cycle of conception. The regimen was aspirin 80 mg daily, heparin subcutaneously 5000 U twice daily, and prednisone 10 mg orally twice daily. Group III women received no autoimmune therapy.

The subsequent prednisone dosage was readjusted according to autoimmune testing every 2 weeks during pregnancy to a maximum dosage of 40 mg per day. The indications for the increased dosage were increasing titers of autoantibodies, antibodies to new epitopes, the reappearance of IgM autoantibodies, or ultrasonic evidence of placental or amniotic fluid compromise. Prednisone was tapered to 10 to 20 mg/day at 24 weeks' gestation and was discontinued at 28 weeks' gestation. Heparin was discontinued at 34 weeks. Aspirin was continued until delivery. All women were monitored in high-risk centers. All women receiving heparin had follow-up clotting studies. Activated partial thromboplastin time and lupus anticoagulant by platelet neutralization procedure[13] and dilute Russell's viper venom time[14] were repeated every 2 weeks during the first trimester and then monthly. Pregnancy losses, fetal growth rates, and fetoplacental Doppler flow kinetics were evaluated monthly by serial ultrasonographic examinations from the first trimester onward. All failed pregnancies were evaluated genetically, immunohistologically, and were tissue-typed (HLA evaluation) by molecular genetic analyses. Analysis of failed pregnancies are the subject of other unpublished reports (Beer and Ober, 1991; Beer and Salafia, 1991).

Statistics. A χ^2 test was used to compare the incidence of autoantibodies and reproductive outcome (success rate, preterm incidence, and large- and small-for-gestational-age incidence); Student's *t* test was used to compare age, gravidity, and parity between study group and multiparous control; and multiple comparison by the Duncan test was used to compare age, gravidity, parity, birth weight, birth height, and gestational age between treatment and nonautoimmune control groups. All analysis was performed with a statistical package for social science version X statistical package on a Dell 386 (Austin, Texas) system computer. Differences were assumed to be significant whenever $p < 0.05$.

Results

Autoantibody profile. The age and gravidity of recurrent spontaneous abortion women and multiparous normal control women were comparable. The ages were 33.3 ± 4.6 and 32.6 ± 4.0, respectively. The incidence of antiphospholipid antibodies in recurrent

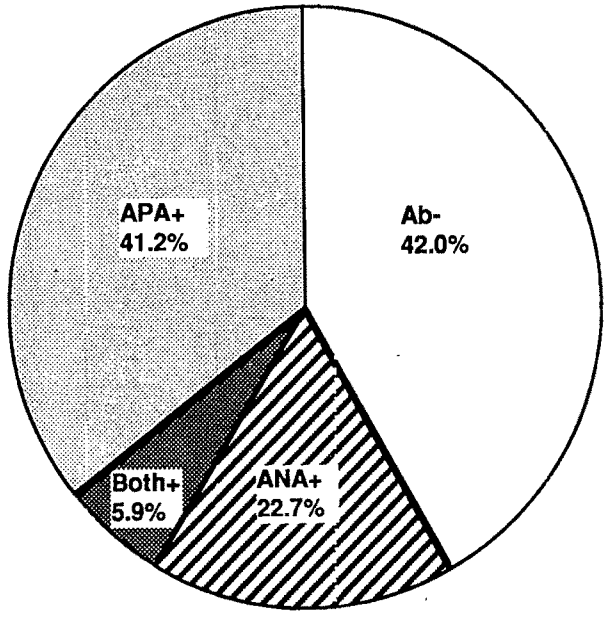


Fig. 1. Autoimmune abnormalities in women with recurrent spontaneous abortion ($n = 270$). *Open area,* women with neither antiphospholipid antibodies *(APA)* nor antinuclear antibodies *(ANA); stippled area,* APA-positive women; *Striped area,* ANA-positive women; *Shaded area,* APA-positive and ANA-positive women.

spontaneous abortion patients (41.2%) was significantly higher than that of the control group (18%) ($p < 0.05$). Incidence of antinuclear antibodies positivity was different between recurrent spontaneous abortion patients (22.7%) and the control group (12.5%) ($p < 0.05$). The autoantibody profiles of the recurrent spontaneous abortion patient population are shown on Fig. 1. When the incidence of positive antiphospholipid antibodies of recurrent spontaneous abortion women is analyzed by the number of previous abortions, there is a stepwise increase in positive antiphospholipid antibody incidence observed with the increasing number of previous spontaneous abortions, but not in age-matched multiparous control by gravidity (same as the number of live-born offsprings) (Fig. 2). Antinuclear antibody incidence showed similar tendencies (Fig. 3).

Alloimmune and autoimmune status of women in treatment and control groups. Age, gravidity, parity, and number of spontaneous abortions of treatment and nonautoimmune control groups are listed in Table I, and there are no differences between groups. Table II provides data to show there were no differences in the alloimmune status of women in any treatment or nonautoimmune control group preimmunization or postimmunization with lymphocytes. All demonstrated an adequate alloimmune response post lymphocyte immunization. Table III lists the autoimmune status of women in all groups. There were no statistically significant differences. This equality between treatment groups provides assurance that the reproductive outcome differences were not explained by more or less severely affected women in any one group.

Reproductive outcome. Pregnancy outcome in all treatment and nonautoimmune control groups is described in Table IV. The nonautoimmune control group that received lymphocyte immunization had a live-birth rate of 84.2%. This is significantly different when compared with the postconception treatment group ($p < 0.05$) and the no-medication group ($p < 0.00001$). The preconception treatment group (group I) had a significantly superior reproductive outcome when compared with the postconception treatment group (group II) ($p < 0.05$) and the no-medication group (group III) ($p < 0.00001$). The postconception treatment group (group II) had a better outcome than the no-medication group (group III) ($p < 0.05$).

Length of gestation. The nonautoimmune control group showed significantly longer gestations than all other groups with autoimmune abnormalities (Table IV, Fig. 4). The length of gestation is increased with autoimmune medication and early onset of medication. When the length of gestation in patients who aborted again after all types of therapy were compared, no significant differences were seen (data not shown). The preconception treatment group infants demonstrated a significantly higher incidence of prematurity than nonautoimmune control group babies ($p < 0.005$). The postconception treatment group also showed a higher incidence of prematurity ($p < 0.05$) when compared with the same nonautoimmune control. In the no-medication group only two women delivered; both infants were premature (Table IV).

Other clinical parameters. Birth weights of infants from all treatment groups were significantly lighter than those of the nonautoimmune control group. No significant differences were seen in babies of any group regarding length, Apgar scores, incidence of intrauterine growth retardation, and largeness for gestational age (Table V). Complications are listed in Table VI; there were no pregnancy complications that appeared unique to any group.

Comment

Our study demonstrates that spontaneous recurrent pregnancy losses are associated with an increasing incidence of autoantibodies to nuclear antigens and phospholipid antigens. The incidence is 34% in women with three recurrent spontaneous abortions and 50% by the fifth pregnancy lost, respectively. This incidence is not seen in control healthy multiparous women or women having their pregnancies electively terminated.[1] There are several possibilities regarding initiation of the au-

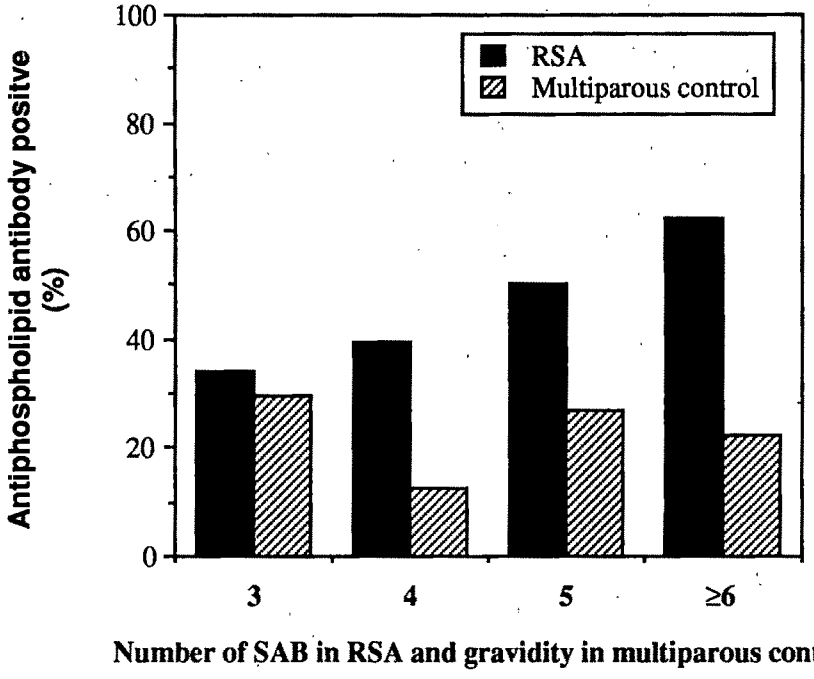


Fig. 2. Incidence of antiphospholipid antibodies in women with recurrent spontaneous abortion *(RSA)* and multiparous control by number of previous spontaneous abortions *(SAB)* (women with recurrent spontaneous abortion) and live-born offspring (multiparous control).

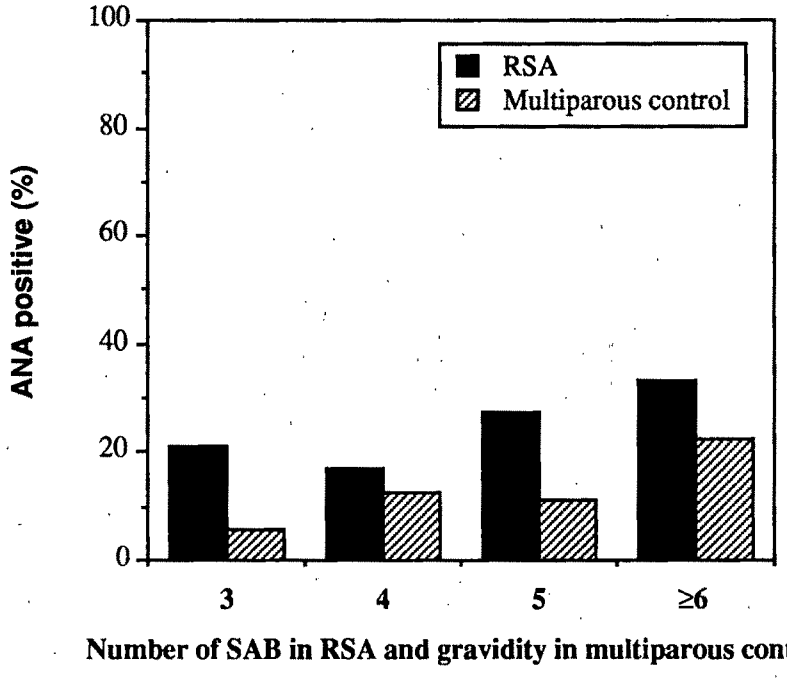


Fig. 3. Incidence of antinuclear antibody *(ANA)* in women with recurrent spontaneous abortion and multiparous control by number of previous spontaneous abortions (*SAB*, women with recurrent spontaneous abortion) and live-born offspring (multiparous control).

toimmunity in patients with recurrent spontaneous abortions: a pregnancy that does not achieve alloimmune protection may stimulate autoimmune response in its mother upon its demise; or in some pregnancies HLA–closely matched couples are autoantigenically unacceptable, that is, they have cell surface molecular features that are known to be associated with autoimmune disorders. In this circumstance they are lost be-

Table I. Age, gravidity, parity, and number of spontaneous abortions in treatment groups and nonautoimmune control group

	Preconception therapy (Mean ± SD)	*Postconception therapy (Mean ± SD)*	*No therapy (Mean ± SD)*	*Nonautoimmune control (Mean ± SD)*	*p Value*
Age	33.6 ± 4.7	32.9 ± 7.2	33.8 ± 4.6	31.2 ± 5.1	NS
Gravidity	4.4 ± 1.8	4.1 ± 1.8	4.8 ± 2.5	4.1 ± 2.0	NS
Parity	0.3 ± 0.8	0.3 ± 0.6	0.4 ± 1.3	0.3 ± 0.7	NS
Spontaneous abortion	3.9 ± 1.6	3.6 ± 1.4	4.0 ± 2.0	3.5 ± 1.6	NS

NS, Not significant.

Table II. IgG maternal antipaternal T- and B-cell antibody levels by flow cytometry before and after lymphocyte immunization

Antibody	*Preconception therapy (Mean ± SD)*	*Postconception therapy (Mean ± SD)*	*No therapy (Mean ± SD)*	*Nonautoimmune control (Mean ± SD)*	*p Value*
Preimmunization					
IgG antipaternal T cell (%)	3.5 ± 11.1	9.9 ± 5.2	3.7 ± 9.8	0.8 ± 1.4	NS
IgG antipaternal B cell (%)	4.9 ± 6.6	5.1 ± 5.7	1.6 ± 4.3	4.3 ± 7.1	NS
Postimmunization					
IgG antipaternal T cell (%)	47.7 ± 35.9	60.7 ± 35.3	56.4 ± 37.5	50.4 ± 41.2	NS
IgG antipaternal B cell (%)	63.5 ± 29.1	60.6 ± 29.7	61.3 ± 33.1	59.6 ± 31.3	NS

NS, Not significant.

Table III. IgG or IgM autoantibody profiles of women in treatment groups and nonautoimmune control group

Autoantibody	*Preconception therapy (%)*	*Postconception therapy (%)*	*No therapy (%)*	*Nonautoimmune control (%)*	*p Value**
ACL	33.1	29.4	12.5	0	NS
APE	21.4	23.5	27.8	0	NS
API	25.2	23.5	12.5	0	NS
APA	23.8	20.6	12.5	0	NS
APS	23.8	29.4	25.0	0	NS
APG	26.2	32.4	12.5	0	NS
ANA	23.8	23.5	38.9	0	NS

ACL, Anticardiolipin; *APE,* antiphosphatidylethanolamine; *API,* antiphosphatidylinositol; *APA,* antiphosphatidic acid; *APS,* antiphosphatidylserine; *APG,* antiphosphatidyl glycerol; *ANA,* antinuclear antibody.

*Significance of the incidence of autoantibodies among pretreatment, posttreatment and no-treatment group.

fore they establish alloimmune recognition. It is quite clear that the establishment of alloimmune recognition in women with autoantibodies to nuclear and phospholipid antigens before their next pregnancy without any further autoimmune treatment is associated with a 90% failure rate. A molecular genetic analysis study of liveborn children is also underway to determine whether surviving children have cell-surface features that cause them to be at a high risk of developing an autoimmune disorder. This study shows that paternal or donor lymphocyte immunization offered to women with recurrent spontaneous abortion is associated with a very high incidence of subsequent pregnancy failure if autoimmune serologic abnormalities are also present in the mother. Our center has consistently tested for both alloimmune and autoimmune abnormalities in women with recurrent spontaneous abortion and is the first to report this finding. The widely varied success rates in lymphocyte-treated women versus control women in centers using immunotherapy for recurrent spontaneous abortion[9, 15] must now be reevaluated on the basis of these new findings. Such collaborative studies are now in progress, and double-blind studies are beginning (unpublished report, Ober and Beer, 1991).

It is of major interest that women with serologic autoimmune abnormalities who have received preconception therapy had significantly better pregnancy outcomes than those whose therapy was started with the first positive β-human chorionic gonadotropin (4 to 5 weeks of gestation at the latest) and with those who

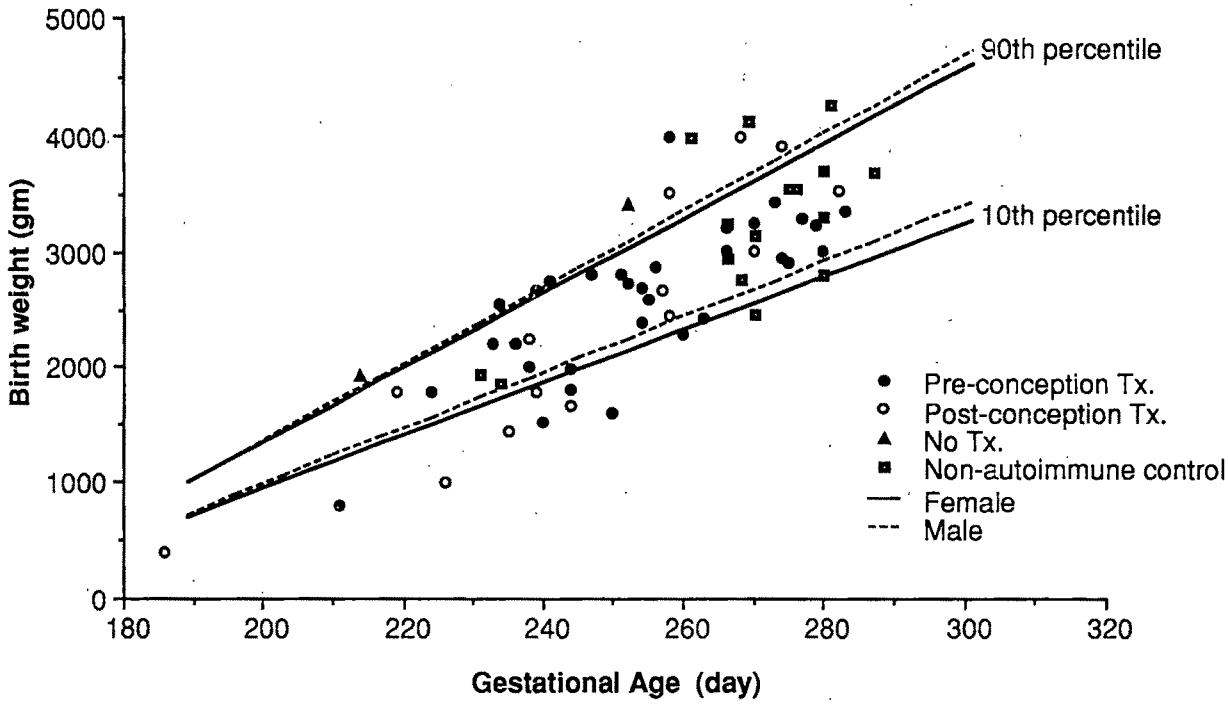


Fig. 4. Reproductive outcome plotting birth weight and gestational age of recurrent spontaneous abortion women according to autoimmune therapy *(Tx)*.

Table IV. Reproductive outcome of treatment groups and nonautoimmune control group

	Preconception therapy	*Postconception therapy*	*No therapy*	*Nonautoimmune control*	*p Value*
Success rate (%)	31/42 (73.8)	15/34 (44.1)	2/18 (11.1)	16/19 (84.2)	Control vs Postconception therapy <0.05 No therapy <0.00001 Preconception therapy vs Postconception therapy <0.05 No therapy <0.00001 Postconception therapy vs No therapy <0.05
Length of gestation (day) (mean ± SD)	253.6 ± 17.7	248.0 ± 25.2	233.0 ± 26.9	268.4 ± 15.6	Control vs Preconception therapy <0.05 Postconception therapy <0.01 No therapy <0.05
Preterm (%)	20/31 (64.5)	10/15 (66.7)	2/2 (100)	2/16 (12.5)	Control vs Preconception therapy <0.005 Postconception therapy <0.01 No therapy <0.05

received no autoimmune therapy at all. If autoimmune therapy is truly associated with this difference, it suggests that immune processes operate far earlier than we had previously suspected. There are several possibilities. There is a large body of data surfacing that autoimmune responses to adhesion molecules that are putatively necessary for the events of implantation and placentation may interfere with these processes. The phospholipids of adhesion molecules that have been studied and shown to be related to placentation are phosphatidylethanolamine and phosphatidylserine. These phospholipids have been shown to serve as adhesion molecules for smooth-muscle and fibroblast cells to fuse and form syncytia.[3, 4] These data suggest that phosphatidylethanolamine and phosphatidylserine molecules may serve in the formation of syncytiotrophoblast from cytotrophoblast. When the configuration of the adhesion molecules are changed experimentally by creating a hexagonal II phase (reversed vesicles with the glue inside them) that is reported to be immunorecognizable, the process of syncytia formation can be stopped.[3, 4, 16] The high incidence of autoantibodies in women with recurrent spontaneous abortion and endometriosis[17] suggests that they are causally related

Table V. Clinical outcomes of pregnancies from treatment groups and nonautoimmune control groups

Clinical outcome	*Preconception therapy (Mean ± SD)*	*Postconception therapy (Mean ± SD)*	*No therapy (Mean ± SD)*	*Nonautoimmune control (Mean ± SD)*	*p Value*
Birth weight (gm)	2679.4 ± 660.8	2441.9 ± 1089.6	2672.0 ± 1052.4	3216.3 ± 715.9	<0.05*
SGA (%)	7/31 (22.6)	5/15 (33.3)	0/2 (0.0)	1/16 (6.3)	NS
LGA (%)	3/31 (9.7)	3/15 (20.0)	2/2 (100)	3/16 (18.8)	NS
Height (cm)	46.5 ± 5.2	50.7 ± 3.6	46.1 ± 5.4	49.6 ± 3.5	NS
Apgar					
5 minutes	7.8 ± 0.9	7.3 ± 1.8	8.0 ± 0.0	8.5 ± 0.5	NS
10 minutes	8.8 ± 0.4	8.8 ± 0.5	9.0 ± 0.0	9.0 ± 0.0	NS

SGA, Small for gestational age; *LGA*, large for gestational age; *NS*, not significant.
*Control vs preconceptional treatment group and postconceptional treatment group.

Table VI. Pregnancy complications in treatment and nonautoimmune control group patients who delivered successfully

Complication	*Preconception therapy (n = 31)*	*Postconception therapy (n = 15)*	*No therapy (n = 2)*	*Nonautoimmune control (n = 16)*
Vaginal bleeding	3	—	1	—
Gestational diabetes mellitus	2	1	—	—
Premature rupture of membrane	3	4	2	1
Preeclampsia	2	—	—	1
Oligohydramnios	—	2	—	—
Placenta abruptio	—	1	—	—
Osteoporosis	1	—	—	—

to early placental organ failure by causing abnormal phase and changes. Studies are now in progress by our group to address this possibility.

The superior success rates of pregnancy experienced by women who were offered preconception treatment must be tempered with the observed and potential complications of this therapy. Although heparin does not cross the placenta[18] and would not be expected to have an adverse effect on the fetus, its therapeutic application is associated with some adverse outcome in approximately one third of pregnancies.[19] In therapeutic doses it may induce osteoporosis.[20] However, the high rate of adverse fetal-neonatal outcome in the heparin-treated patients could be explained by the presence of maternal comorbid conditions.[21] Recently it has been reported that the rate of prematurity, abortion, stillbirth, neonatal death, and congenital abnormality of individuals who received a high-dose heparin regimen throughout pregnancy were similar to in the normal population.[22] In this study patients received a mini-dose of heparin until 34 weeks' gestation. Three patients (3.9%) in the preconception treatment group had mild vaginal bleeding episodes during the course of heparinization. It has been reported that 2% of the patients who received heparin during pregnancy had significant antepartum vaginal bleeding.[22] An incidence of heparin-associated thrombocytopenia is reported as 10%[23]; however, in our series no patient had thrombocytopenia while on heparin. One patient with the preconception minidose heparin treatment had osteoporosis. We recommend bone densitometry after delivery to further evaluate this risk.

Low-dose aspirin therapy is reported to be safe for the fetus and mother. A dose of 60 to 80 mg of aspirin daily during pregnancy selectively inhibits maternal platelet cyclooxygenase without affecting neonatal platelet aggregation or pulmonary circulation. Maternal platelet thromboxane B2 production in response to adenosine diphosphate or collagen was reduced 98% by the 80 mg dose after 1 week of therapy.[24] The superior success rate observed with preconception treatment may be partly explained by this.

What constitutes an autoimmune abnormality in women with recurrent spontaneous abortion is not yet defined or agreed on. However, there is evidence that trophoblastic antigenic stimuli during pregnancy may induce autoantibodies, including antiphospholipid antibodies.[25] How often the abnormalities discussed in this study are seen in normally reproducing women before and during pregnancy is unknown and is currently under study by our group. It is becoming clear that recurrent spontaneous abortion women with autoimmune abnormalities and no symptoms of autoimmune disease who have entered our study early and have been

observed for more than 12 years have not had autoimmune symptoms or diseases. Studies are in progress to determine whether autoimmune preconception therapy alone without any lymphocyte immunotherapy, allows more appropriate fetal-embryonic trophoblast antigen presentation in the prospective mother by the spontaneous development of the correct alloimmune recognition response that attends a normal pregnancy. This study applies most specifically to women who lose embryos repeatedly and very early after in in vitro fertilization programs and embryo transplantation. Multicenter prospective clinical studies are needed regarding the immune consequences to the mother of recurrent spontaneous abortion and its appropriate therapy.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

Dr. James R. Scott, Salt Lake City, Utah. Potential immunologic aspects of recurrent spontaneous abortion is a timely topic that has been well publicized in both the medical and lay literature, but it remains a confusing and controversial area clearly in need of well-controlled studies. The purpose of this study was to determine whether treatment early in pregnancy with aspirin, prednisone, and heparin was beneficial in recurrent abortion patients of presumed autoimmune causes. In my opinion the present study requires cautious interpretation for several reasons.

A battery of autoimmune assays was performed on each patient (six antiphospholipids, IgG, IgM, IgA subclasses of each, and an antinuclear antibodies), and at least one positive test was presumably the basis for an autoimmune diagnosis. However, the actual results of the tests are not given. If a mean +3 SD as normal, there is a 17% chance that at least 1 of the 19 laboratory tests will be abnormal but clinically insignificant in any given normal individual. For example, there is a 10%

to 20% false-positive rate of antinuclear antibodies in normal pregnant women.[1] To my knowledge the only proven autoantibodies associated with recurrent pregnancy loss are lupus anticoagulant and anticardiolipin, and elevation of IgM and IgA values may be nonspecific and unrelated to reproductive problems. We have found that using +3 SD as the cutoff between normal and abnormal levels for anticardiolipin and other antiphospholipid antibodies yields an unacceptably high number of weak positives and overdiagnosis of this condition in women who actually have successful pregnancies without treatment.[2] I suspect that these factors contributed to the overall 68% incidence of autoantibodies in this population of recurrent spontaneous abortion patients because this is much higher than has been reported by other groups.

Any study of pregnancy-loss patients should clearly define the patient selection criteria to show that the groups are truly comparable. How were the patients randomized, and was the study blind? If not, the potential for bias is high and jeopardizes the results and conclusions. I was unable to determine how ovulation was detected, how it was decided which patients would be placed in each group, over what period of time the study occurred, what physicians participated, the total number of patients treated who did not conceive, how long treatment was continued in those women, and how accurate follow-up information was obtained. If heparin were started 48 hours after ovulation, this treatment for group I was not preconceptual but rather preimplantation. It is unclear to me exactly how the drug regimen was monitored in each patient, the criteria for raising the prednisone dose to as high as 40 mg per day, and what the dose relationship was to the pregnancy outcome. The cost of these diagnostic tests is significant, and repeating all tests every 2 weeks would be very expensive from a practical standpoint. Anticardiolipin levels are often not altered by treatment, and we have found little relationship between live birth rates and lupus anticoagulant levels. It also seems doubtful that meaningful Doppler flow kinetics could be monitored in the first or early second trimester, and no data are shown.

On the basis of their results, the authors advocate preimplantation treatment with aspirin, prednisone, and heparin. However, their 73.8% success rate is no higher than that reported by a number of investigators for well-defined antiphospholipid syndrome patients with recurrent pregnancy loss who began treatment with low-dose aspirin and either prednisone or heparin alone after pregnancy was diagnosed.[2-4] If both of the authors' treatment groups are combined, the live-birth rate is only 62%, raising the possibility that this drug combination is actually less effective. According to our statistician, χ^2 tests should not be used when the numbers are too small, as they are in some cases. For example, mean values and statistical comparison of the length of gestation, birth weight, height, and Apgar scores in Tables IV and V are rather meaningless when there are only two patients in the group with no treatment.

The authors rightly comment on potential serious complications when corticosteroids and heparin are combined during pregnancy. We have recommended against this treatment in the absence of evidence that it is better than prednisone or heparin alone, and we know of several patients treated with both drugs who developed severe osteopenia and vertebral or hip fractures. Therefore I believe that the effectiveness and safety of this regimen must be clearly shown before it is widely adopted. Finally, because this study as experimental, was it approved by the Institutional Review Board, and did the patients pay for these tests and treatments?

Dr. Beer and his colleagues have been major contributors to innovative research in reproductive immunology over the past decades. I enjoyed reading the paper and encourage them to continue their investigation of recurrent pregnancy loss.

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DR. JOHN G. GIANOPOULOS, Maywood, Illinois. I would like to thank the Association for the privilege of discussing this most interesting and timely article. It is estimated that 20% to 30% of all recognized pregnancies end in spontaneous abortion. Patients experiencing ≥3 repetitive losses with no live-born children account for 1% of all pregnancies. These unfortunate pregnancy outcomes may have a number of causes. Anatomic malformations, hormonal deficiencies, infectious pathogens, parental genetic abnormality, and immunologic causes have all been implicated in this pathologic reproductive entity. Recently there has been much research directed at immunologic causes for repetitive pregnancy loss, particularly the phospholipid antibody syndrome. Dr. Kwak et al. have focused their work on those patients suffering immunologic reproductive failure.

All patients entered into their analysis were screened for all the known causes for recurrent abortion, and only those patients with immunologic abnormalities were included in this study. Patients in this study were all determined to have alloimmune abnormality with low levels of paternal blocking antibody. They were all treated with leukocyte immunization. The experimental group consisted of 94 patients with autoantibodies

Table I. Postpregnancy therapy

Investigator (yr)	*Liveborn/total pregnancies*	*Mode of therapy*
Lubbe and Liggins (1984)	7/8	Steroid, ASA, and heparin
Farquhansen et al. (1984)	2/2	Steroid and ASA
Gleicher and Friberg (1985)	5/8	Steroid
Branch et al. (1985)	5/8	Steroid and ASA
Rosove et al. (1990)	14/15	Heparin alone
Carlson et al. (1991)	43/51	Steroid and ASA
TOTAL	76/92 83%	
Kwak et al. (1991)	15/34 44.1%	

ASA, Acetylsalicylic acid.

to phospholipid. Patients were assigned to prepregnancy therapy, postconception therapy, and no-therapy groups. All groups were similar in their demographics, with similar reproductive characteristics. Patients receiving prepregnancy therapy with prednisone, aspirin, and heparin demonstrated a significantly better outcome of live-born neonates than the other groups, 73% versus 44% and 11%, respectively.

This study raises a number of interesting questions. Is there an advantage to successful outcome in pretreating patients with thrombotic and immune therapy rather than initiating therapy at the onset of a recognized pregnancy? On review of the existing literature, all with postpregnancy initiation of therapy, there appears to be no advantage to prepregnancy therapy because the successful outcome equals 80%, which is not significantly different from Dr. Kwak's pretreatment group. In fact, the current study's postpregnancy treatment group demonstrates a significantly different outcome than would be expected from previously published reports (Table I). The previously reported series were not controlled for alloimmune causes. Could this explain the differences in outcomes? Each patient in Dr. Kwak's analysis, however, was treated for alloimmune deficiency and demonstrated normal blocking antibody. This leads me to assume that alloimmune incompetence cannot explain this obvious difference.

It is known that steroids may lead to adverse reactions related to the length of exposure; if it is assumed there is no benefit from pretreatment, this strategy may unnecessarily expose patients to potentially dangerous therapy for longer than is needed. The authors report no adverse reactions to therapy. However, in analyzing their data, I note that complications of gestational diabetes and pregnancy-induced hypertension (both steroid-related complications) were higher in the pretreatment group 4 (12.9%) of 31 versus 1 (6.7%) of 15 in the postpregnancy treatment group. Could the longer exposure to steroids have led to this observation? The authors do not report the average time interval from the initiation of therapy to the onset of pregnancy in the prepregnancy therapy group.

Patients with recurrent abortion caused by antiphospholipid antibodies undergoing therapy have been reported to show a 50% to 60% incidence of prematurity complications. The data in this study also demonstrate this observation, with a 64% prematurity rate in the treated groups. It is important to keep this in mind when caring for these patients and to take precautions in management for the potential of preterm labor.

I pose the following questions to the authors:

1. The groups included in the analysis are of unequal size. How were patients assigned to therapeutic groups? Were patients randomly assigned, or were clinical factors used in assignment to experimental groups? Could this have affected outcome?
2. What factors, if any, could explain Dr. Kwak's observation of a poorer outcome in the postpregnancy therapy group when compared with previously published reports?
3. What was the average interval from the onset of therapy in the prepregnancy treatment group to the onset of pregnancy? Do the authors feel that this interval explain the slightly higher incidence of steroid-related complications in the prepregnancy treatment group?
4. Do the authors believe that the antithrombotic effect of salicylate and heparin has a critical effect and that prepregnancy steroid therapy may not be needed?

Future research will be needed to answer the question as to whether pretreatment with aspirin or heparin alone improves outcome.

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Dr. Michael R. Caudle, Knoxville, Tennessee. Why do the authors continue to do HLA typing to diagnose

alloimmune failure when it does not clearly identify a population that has pregnancy loss?

Dr. Kwak (Closing). This preconceptional treatment study for women with recurrent spontaneous abortion was initiated because preconception therapy in autoimmune infertile women who became recurrent spontaneous abortion patients through in vitro fertilization or gamete intrafallopian transfer were having a better reproductive outcome than the classical recurrent spontaneous abortion patients who heretofore had therapy initiated at the onset of a positive β-human chorionic gonadotropin. This is not a double-blind study. Sequential patients were allocated into the three different subgroups after a comprehensive physician-patient consultation to ensure that patients in the various subgroups were comparable regarding age, gravidity, and laboratory abnormalities.

β-human chorionic gonadotropin testing was used to determine the onset of pregnancy and the onset of postconception therapy. This was done from day 25 through day 30. Women were instructed to begin therapy at the time of a positive β-human chorionic gonadotropin.

Ninety-four of 111 patients in this study established their pregnancies within 6 months. The remaining 17 did not establish a pregnancy during the study period. The age, gravidity, and alloimmune and autoimmune parameters in these women were not significantly different from those who established pregnancies.

Doppler ultrasonographic evaluation was done on all women sequentially during pregnancy. These findings are the subject of a separate report.

The enzyme-linked immunosorbent assay is used to detect antiphospholipid antibodies. Sixty healthy, nonpregnant women were used as controls. Nonspecific background bindings were subtracted. The value at mean ± 3 SD corresponds in optical density to the moderate positivity reported by Harris using control sera provided by him. Because patients are screened every 2 weeks and sera from prior testing is repeated with subsequent testing, we have reduced our interassay coefficients of variability to a low level, which provides assurance that the 3 SD value is reliable and real.

In a review of the literature regarding osteoporosis and heparin therapy, 119 patients are available for analysis. No case of heparin-induced osteoporosis was found when the dosage was ≥10,000 U per day. Six of 10 women receiving a therapeutic heparin dosage had osteoporosis. All of our patients received 10,000 U of heparin daily. We report one case of mild osteoporosis that resolved postpartum. This patient experienced a 25% density loss. She is now pregnant a second time. We advocate bone densitometry assay for women receiving both heparin and prednisone, not minidose heparin alone.

Previous reports regarding human leukocyte antigen sharing between recurrent spontaneous abortion couples are still controversial. Maternal antipaternal antibody before and after lymphocyte immunization correlates with the number of HLA antigens shared, specifically at HLA-DR and HLA-DQ. The greater the numbers shared, the lower the postimmunization responses and the greater number of pregnancy losses after immunization. In couples with excessive DR/DQ antigen sharing we found the reproductive outcome to be poor when compared with couples who do not share these antigens. In addition, when donor immunization was indicated, tissue typing was done for selection of the most appropriate donor.

Lack of alloimmune recognition before and after paternal or donor lymphocyte immunization is defined by a flow cytometer crossmatch developed by us. This crossmatch is very sensitive and can document a maternal antipaternal anti–T- and –B-cell response very early in pregnancy. The crossmatch by microcytotoxicity will be positive in only 50% of these pregnant women and is a test not sufficiently sensitive to be useful clinically. In a study by Kiprov,[1] 72 women with successful pregnancies were compared with 141 women with recurrent abortions and with 72 heterosexual males whose cell and sera were paired in this assay. Pregnant women had crossmatch values six times greater than women with recurrent spontaneous abortion or the male controls.

I am glad to have received so many interesting and challenging questions. This topic is a clinical one of continuing controversy. Fortunately, newly developed immunologic assays allow a critical analysis of alloimmune and autoimmune responses during pregnancy. It is becoming clear that a fetus must signal the maternal immune system to deal with it in a manner unlike that involved in an infectious response. It also is becoming clear that fetuses who do not stimulate an alloimmune response in their mothers are subject to the elicitation and expression of subtle autoimmune responses that may be detrimental to placental development. Certainly double-blind multicenter trials are now necessary in the further dissection of the problem of recurrent spontaneous abortion.

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The Green Bay cesarean section study

II. The physician factor as a determinant of cesarean birth rates for failed labor

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OBJECTIVE: Our study was designed to develop a profile of specific labor management characteristics generally used by physicians with low versus those with high rates of cesarean sections in the care of nonprogressive labor in nulliparous patients.
STUDY DESIGN: A 4-year retrospective data set was used to analyze all patients with nonprogressive labor cared for by 11 board-certified obstetricians and gynecologists practicing full-time at two Green Bay hospitals. Variations in labor management are analyzed and tested for their effect on the rate of cesarean section for failure of labor to progress.
RESULTS: Cesarean section in nulliparous women for nonprogressive labor varied from 4.3% of all deliveries in the low group to 12.3% in the high group. Through multivariate analysis we developed a profile of specific labor management characteristics used by physicians with low versus those with high rates of cesarean section.
CONCLUSION: These techniques can be used to definitively identify management strategies that result in a decrease in cesarean rates for nonprogressive labor. (AM J OBSTET GYNECOL 1992;166:1799-1810.)

Key words: Cesarean section, nonprogressive labor

The United States cesarean section rate has received increasing attention and concern after its rise from 5% in the early 1970s to 15% in the late 1970s and approximately 25% in 1988. The current rate is viewed as a national disgrace by some[1] and as about right by others,[2] demonstrating the divergence of opinion regarding the optimal cesarean rate. Increasing concern with a 15% cesarean rate led to the convening of a consensus development conference in 1980. The major conclusion of the conference members was that the rise of the cesarean rate to 15% was a matter of concern and that the goal of clinical obstetricians everywhere should be to contain this trend while maintaining good maternal and perinatal outcome.[3] The ensuing further increase demonstrates the difficulty encountered in translating research findings, guidelines, and recommendations into clinical practice.

We have been concerned about cesarean rates associated with "modern" perinatal care. In our previous Green Bay cesarean section study[4] individual physician cesarean rates ranged from 5.6% to 19.7%. Cesarean rates for physician groups ranged from 9.8% to 18% with proportional differences for the four major indicators for cesarean section: repeat cesarean, failure of labor to progress, breech presentation, and fetal distress. The variances in cesarean rates among individual and groups of physicians were not attributable to patient obstetric risk factors, socioeconomic status, service status, or duration of the physician's practice. Higher cesarean rates did not result in better newborn outcome. Individual physician practice style was the only apparent determinant of cesarean rates. It was concluded that the current cesarean rates could be substantially reduced without sacrificing fetal and newborn safety.

Presently we are attempting to identify those specific variations in practice style for each of the major indicators for cesarean section that result in variable cesarean rates in similar patient populations. This report analyzes those variations in the management of nonprogressive labor (dystocia).

Repeat cesarean section is the most common indication for all cesarean deliveries in the United States. Nonprogressive labor is the most common indication for cesarean section in nulliparous women. It is apparent that many physicians do not encourage women who have experienced nonprogressive labor initially to attempt a vaginal birth in subsequent pregnancies. Thus this self-perpetuating cycle, beginning with ce-

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Table I. Biologic and socioeconomic patient characteristics that may influence cesarean rates and newborn outcomes

Biologic
1. Gestational age
2. Maternal height
3. Maternal weight and weight gain
4. Birth weight
5. Head circumference
Socioeconomic
6. Insurance status
7. Maternal smoking
8. Race
9. Lamaze training

sarean section for failure to progress (dystocia) in the nulliparous labor, should be averted, where possible, to reduce the rates of cesarean section.

Material and methods

For this study all cesarean deliveries performed for nulliparous, term, and vertex presentation pregnancies were analyzed when the primary indication for cesarean section was listed by the obstetrician as either "failure to progress" or "cephalopelvic disproportion"; our 3-year review of all cesarean deliveries from 1986 to 1988 was used as the data base.[4] We added a fourth year of data, 1989, collected in the same fashion. Also included in this 4-year analysis are all women laboring for the first time after one or more previous cesarean sections. The study included all such cesarean sections performed by 11 board-certified obstetricians and gynecologists practicing exclusively and full-time at two Green Bay hospitals.

A second study group included the same detailed analysis of all operative vaginal deliveries (forceps or vacuum extractions) for similar patients done by the same physicians when the second stage of labor was prolonged for >2 hours or the physician listed the indication as "failure to progress."

Randomized controls consisted of spontaneous vaginal deliveries from the 4-year analysis (nulliparous, term, and vertex presentations). For each cesarean delivery the next spontaneous vaginal delivery was chosen as a control.

From the 9480 deliveries occurring during the 4-year data collection period, we identified 766 deliveries meeting those criteria and divided them into the following groups: group 1, cesarean deliveries for nonprogressive labor, $n = 212$; group 2, forceps or vacuum deliveries for nonprogressive labor, $n = 143$; group 3, spontaneous vaginal deliveries (control), $n = 411$.

Each study group was analyzed for patient characteristics that influence cesarean delivery rates to ascertain distribution among the 11 physicians (Table I). An estimation was made for each physician of the percentage of his or her practice that consisted of nulliparous, term, vertex pregnancies based on the profile provided by the randomized control vaginal deliveries and the known values in their entire cesarean section population. Thus we could calculate for each physician a rate of cesarean section for failed labor in nulliparous, term, vertex, singleton pregnancies (Table II).

The physicians were placed into low, medium, and high groups based on their rate of cesarean delivery for failure to progress (Table II). Also listed are comparable rates of operative vaginal deliveries. For comparison, Table III lists the overall cesarean rate and the number of repeat cesarean deliveries for each physician. The possible variations in labor management by the groups of physicians, which are analyzed in detail, are listed in Table IV.

These maternal and neonatal outcomes were analyzed: 1- and 5-minute Apgar scores, need for resuscitation, neonatal intensive care unit admissions, need for ventilatory support, morbidity, mortality, and length of stay.

We analyzed the data by the use of "Proc freq" and "Proc GLM" in the Statistical Analysis Systems. Relevant continuous variables were subjected to two-way analysis of variance to examine interaction between physician groups and mode of delivery. Where the data were frequencies, two-way tables of frequencies were analyzed by χ^2. The Wilcoxon test of significance was used for nonparametric data. Separate analyses were performed for cesarean sections (group 1), forceps and vacuum extractions (group 2), and spontaneous vaginal deliveries (group 3).

Results

Overall cesarean rates of individual physicians varied from a low of 5.0% to a high of 19.5% (Table III) with a range of 9.4% to 16.4% by groups of physicians. Cesarean section for failure to progress varied from 2.7% of all primary deliveries to 16.0% for individual physicians with a range of 4.3% to 12.3% by groups of physicians (Table II). Socioeconomic characteristics (Table V) did not vary between physician groups or mode of delivery. The one exception, with no apparent explanation, involved a greater percentage of Lamaze-trained patients in the group requiring forceps- or vacuum-assisted vaginal deliveries.

Maternal and fetal biologic characteristics (Table VI) did not differ between physician groups. Of greatest importance, the average newborn birth weights and head circumferences were the same among physician groups. As expected, all biologic characteristics varied significantly between modes of delivery. Cesarean de-

Table II. Primary deliveries: Singleton, vertex presentation, term gestation

Group	Physician No.	No. of deliveries	Cesarean sections for nonprogressive labor		Group mean (%)	Forceps or vacuum extractions for nonprogressive labor		Total operative rate for nonprogressive labor (%)	Group mean (%)
			No.	%		No.	%		
Low	1	292	8	2.7	4.3 (47/1096)	19	6.5	9.2	
	2	196	9	4.6		13	6.6	11.2	9.9
	3	204	10	4.9		7	3.4	8.3	
	4	404	20	5.0		22	5.4	10.4	
		1096	47						
Medium	5	212	14	6.6	7.8 (85/1093)	16	7.5	14.1	
	6	211	16	7.6		13	6.2	13.8	13.5
	7	385	30	7.8		19	4.9	12.7	
	8	285	25	8.8		14	4.9	13.7	
		1093	85						
High	9	191	18	9.4	12.3 (80/652)	28	14.6	24.0	
	10	199	20	10.0		19	9.5	19.5	19.8
	11	262	42	16.0		2	0.8	16.8	
		652	80						

Table III. Overall cesarean rate and use of repeat cesarean section by groups of physicians (all singleton fetuses)

Group	Physician no.	Total no. of deliveries	Total no. of cesarean sections	No. of repeat sections	Overall cesarean rate (%)	Group mean rate (%)
Low	1	617	37	3	6.0	9.4
	2	808	78	29	9.8	
	3	953	76	23	8.0	
	4	1189	144	50	12.1	
		3567	335			
Medium	5	661	88	33	13.3	16.4
	6	578	88	26	15.2	
	7	1347	224	103	16.6	
	8	821	160	52	19.5	
		3407	560			
High	9	625	86	23	13.8	15.0
	10	893	106	25	11.9	
	11	988	185	71	18.7	
		2506	377			

liveries included larger newborns with larger head circumferences born to shorter and heavier women at greater gestational ages.

Epidural use is very uncommon in Green Bay. Only five laboring patients received intrapartum epidural anesthetics, and three of them had failure to progress and underwent cesarean delivery.

Significant differences were noted between physician groups in the peak dose of oxytocin use (Fig. 1) and the cervical dilatation at which both oxytocin was initiated and the cesarean section was ultimately performed (Table VII). In a significantly greater percentage of patients in the low group complete dilatation was achieved before cesarean delivery, and physicians in the low group allowed significantly longer second-stage labors (although this contribution was primarily in patients who were ultimately delivered by forceps or vacuum). Physicians in the low group gave oxytocin augmentation to a significantly greater percentage of their spontaneous labor patients before they resorted to cesarean section for nonprogressive labor (82% vs 58% for the combined medium and high groups, $p < 0.01$). Cephalopelvic disproportion or suspected macrosomia were commonly cited by high and medium cesarean rate physicians when oxytocin augmentation was omitted in their management of nonprogressive labor. In the low group only 6 of 34 spontaneously laboring patients did not receive oxytocin before undergoing cesarean delivery. Also noted was a tendency ($0.05 < p < 0.10$) for physicians in the low group to

Table IV. Variables studied in labor management

1. Time distributions of:
   a. Cesarean delivery
      Morning (6 AM to noon)
      Afternoon (noon to 6 PM)
      Evening (6 PM to midnight)
      Night (midnight to 6 AM)
   b. Admission
   c. Rupture of membranes
2. Induction of labor by artificial rupture of membranes
3. Oxytocin
   a. Cervical dilatation at time of augmentation
   b. Maximum dose
   c. Duration of use
4. Use of epidural anesthesia
5. Frequency of indicated forceps or vacuum delivery
6. Cervical dilatation at time of cesarean section
7. Duration of first stage of labor at time of cesarean section
8. Duration of second stage of labor at time of forceps or vacuum delivery
9. Duration of second stage of labor at time of cesarean delivery

administer oxytocin for longer periods of time and to allow a greater duration of membrane rupture before delivery (Table VII).

There were no significant differences between physician groups in the percentage of patients with labor induced, the length of first-stage labor, or the time of day cesarean delivery was performed.

For neonatal and maternal outcomes, logistic regression demonstrated no differences in Apgar scores (Table VIII), length of stay, neonatal intensive care unit admission, respiratory support, or significant morbidity between physician groups. There were no neonatal or maternal deaths in this study population. Maternal morbidity was quite rare with no differences between physician groups. All six cases of endometritis occurred in patients with cesarean section.

Comment

O'Driscoll et al.,[5] at the Dublin Maternity Hospital, demonstrated that active management of labor enables them to safely minimize the need for cesarean section for the diagnosis of dystocia. Turner et al.[6] reported similar results, with a cesarean rate of 5.6% among nulliparous patients given an oxytocin regimen of 6 to 40 mU/min, with increases at 15-minute intervals. Fraser et al.[7] found a substantial increase in cesarean births during convenient evening hours; however, when corrections were made for the unexpected temporal variations in the proportion of patients in prolonged labor, the disproportion was minimal, accounting for only 15% of the cesareans for dystocia. Neuhoff et al.,[8] DeRegt et al.,[9] and Taylor,[10] as well as others, has demonstrated that private patients in university teaching hospitals are subjected to cesarean section more readily for failure to progress in labor than are "clinic" patients. Gould et al.[11] noted a 76% higher cesarean rate when considering patients with high versus low family incomes. Substantially higher cesarean rates have been reported with the use of epidural anesthesia.[12]

Many authors have observed the lack of consistency between research findings and clinical practice. Lomas et al.[13] answered their question, "Do practice guidelines guide practice?" in the negative. Commenting on the poor relationship between practice behavior and published literature, Greer[14] demonstrated that the need for change must come at the local level rather than externally. Similarly, Stafford[15] believes that " . . . formal programs aimed at modifying practices within individual hospitals appear to be the most successful . . . " for controlling rising cesarean rates. Porreco,[16] also emphasizing the local level, recommended the following for each hospital: an individual physician designated as the cesarean section monitor, coalitions to manage labor, and tort reform measures of proved effectiveness similar to those enacted in California.

All of these liken the high cesarean rate to Mark Twain's comment on the weather: "Everybody talks about it, but nobody does anything about it." Actually, the rising concern has galvanized some individuals and groups into action, which is currently in the planning stages with no results to date. Examples include a joint New York task force[17] consisting of members of the New York State Health Department, the American College of Obstetricians and Gynecologists, and the Hospital Association of New York State. Similarly, the Maryland Hospital Association has embarked on a research project: "Evaluating the appropriateness of care: a study of cesarean section rates."[18]

To reduce the cesarean rate in nonprogressive labor, we must identify those management techniques that are effective in achieving that goal. Our data support the notion that physicians may achieve lower rates of cesarean section for failure to progress (4.3% vs 12.3%) by giving augmentation to more patients in spontaneous labor and giving higher doses of oxytocin for longer periods of time but waiting until the truly active phase of labor (later cervical dilatation) before initiating oxytocin or resorting to cesarean section. High and medium cesarean rate physicians were reluctant to give augmentation to some patients because of perceived cephalopelvic disproportion or macrosomia, in spite of the inability to diagnose cephalopelvic disproportion without an adequate trial of labor[19, 20] and the known

Table V. Socioeconomic characteristics by population means, groups of physicians, and mode of delivery

	Insurance status				
	Private (%)	*Public assistance (%)*	*Maternal smoking (%)*	*Nonwhite race (%)*	*Lamaze training (%)*
Patient population (mean)	90	10	20	0.7	90
Physician group					
Low	88	12	23	0.9	89
Medium	89	11	20	0.6	89
High	93	7*	20	0.5	91
Mode of delivery					
Cesarean section	91	9	20	0.5	89
Assisted vaginal	94	7	16	1.5	98†
Spontaneous vaginal	88	12	24	0.5	87

*$p = 0.10$.
†$p < 0.05$.

Table VI. Biologic characteristics by population mean, groups by physicians, and mode of delivery*

	Gestational age (wk)	*Infant weight (gm)*	*Maternal weight (lb)*	*Maternal weight gain (lb)*	*Maternal height (inches)*	*Infant head circumference (cm)*
Patient population (mean)	40	3653	176	33	65	35
Physician group						
Low ($n = 211$)	40	3688	177	34	65	35
Medium ($n = 335$)	40	3672	179	33	65	35
High ($n = 220$)	40	3612	173	32†	65	35
Mode of delivery						
Cesarean section ($n = 212$)	40.3†	3837†	187†	35†	64†	36†
Assisted vaginal ($n = 143$)	39.7	3649	171	32	65	35
Spontaneous vaginal ($n = 411$)	39.9	3487	171	32	65	35

*Least-squares mean values.
†$p < 0.05$.

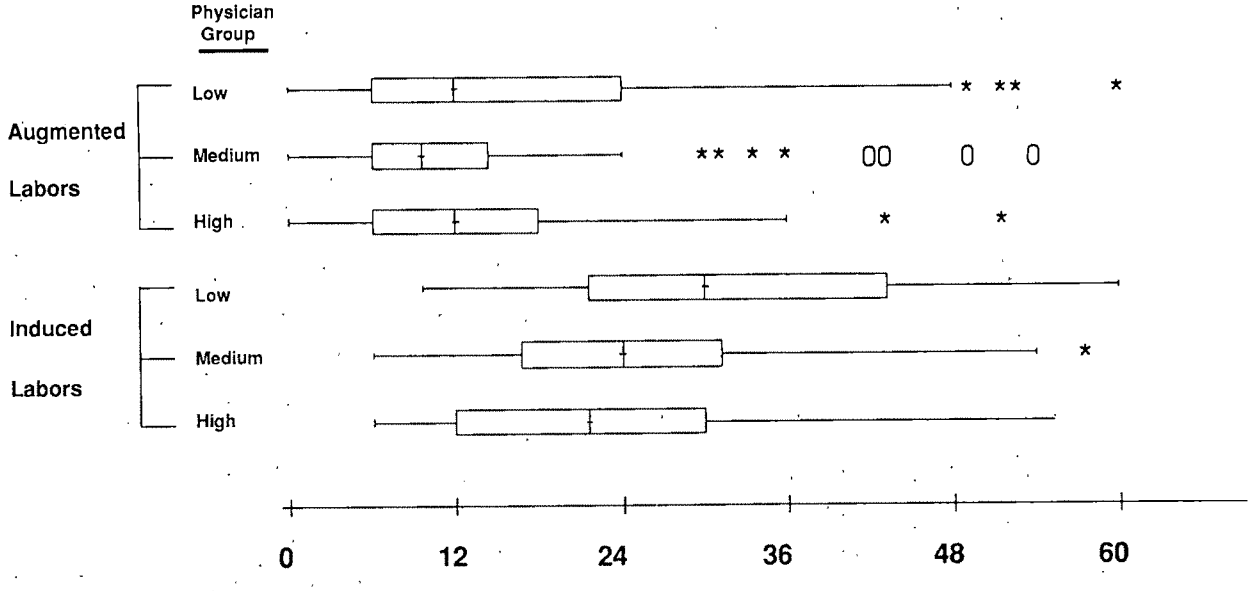


Fig. 1. Distribution of peak oxytocin doses (milliunits per minute). *Asterisk*, Possible outliers; *0*, probable outliers. Outliers are included in calculation of mean values and standard deviations.

Table VII. Management differences between groups of physicians

Physician group	Oxytocin use*					Length of labor		Stage 2 length for cesarean sections (hr)	Stage 2 length for assisted vaginal deliveries (hr)
	Peak dose (mU/min)	Duration (min)	Beginning cervical dilatation (cm)	Patients		Stage 1 (hr)	Stage 2 (hr)		
				Augmentation (%)	Induction (%)				
Low	18.3	315	5.3	43	16	11.6	2.34	3.2	3.0
Medium	12.0	243	4.0	30	15	9.9	2.25	3.4	2.5
High	11.9	275	4.7	31	21	11.5	2.28	3.2	2.8
Significance†	$p = 0.001$	$p = 0.06$	$p = 0.01$	$p = 0.04$	NS	NS	$p = 0.001$	NS	$p = 0.06$

NS, Not significant.
*Oxytocin use and length of labor and duration of membrane rupture for all patients in the study ($N = 766$).
†Wilcoxon test of significance.

Table VIII. Apgar scores at 1 and 5 minutes by physician groups and mode of delivery ($N = 766$)

Mode of delivery	Apgar score	Apgar score at 1 min			Apgar score at 5 min			Statistical significance
		Low physician group (%)	Medium physician group (%)	High physician group (%)	Low physician group (%)	Medium physician group (%)	High physician group (%)	
Cesarean	0-3	12	5	6	0	0	0	NS
	4-6	21	19	18	4	1	0	
	7-10	67	76	76	96	99	100	
Assisted vaginal	0-3	10	0	6	0	0	0	NS
	4-6	21	18	21	6	4	3	
	7-10	68	82	73	94	96	97	
Spontaneous	0-3	3	3	2	0	0	0	NS
	4-6	8	12	10	3	1	0	
	7-10	89	86	87	97	99	100	
Total for all modes of delivery†	0-3	5 (10)	3 (9)	4 (8)	0 (0)	0 (0)	0 (0)	NS
	4-6	13 (27)	13 (44)	14 (30)	2 (5)	1 (4)	1 (1)	
	7-10	82 (169)	84 (279)	83 (180)	97 (200)	99 (323)	99 (212)	

NS, Not significant.
*Values in parentheses are numbers.

inaccuracy of fetal weight[21] estimations, whether by ultrasonography or clinical palpation. This reluctance may have been fostered by the *ACOG Technical Bulletin* on "Induction and augmentation of labor"[22] in force during most of the period of this study, to wit: "After cephalopelvic disproportion and macrosomia have been ruled out. . . ." Perhaps the time has come to abandon the term cephalopelvic disproportion as it relates to nonprogressive labor. Certainly the extensive experience with vaginal birth after cesarean section[23] demonstrates the futility of thinking that we can identify that entity. Our findings agree with a recent statement made by Porreco[16] that in many cases failure to progress means failure to wait. There is ample suggestion from our data that the lower cesarean rate physicians are more patient in their management of slowly progressing, nulliparous patients. These management techniques, which reduce the need for cesarean delivery, can be utilized in similar patient populations with equal newborn outcomes.

Given the analysis of our data leading to these conclusions, we ask ourselves: Can it be that simple? Do "modern" oxytocin dose regimens generate "modern" cesarean delivery rates? Preliminary to our analysis of historic information regarding cesarean rates for oxytocin, we make a presumption that the pelvic capacity of women in the United States has not decreased and that newborn size has not significantly increased during the last 20 years. Meanwhile, cesarean rates for dystocia have increased fourfold to eightfold during that time period. The review of historic information suggests the correctness of our conclusion, which is as follows: To reduce the need for cesarean section in nulliparous patients, administer more oxytocin to more patients for longer time periods.

What factors have caused the decrease in dose, duration, and frequency of use of oxytocin during the time period of escalating cesarean rates for nonprogressive labor? Haverkamp et al.[24] found an increase in cesarean rates for dystocia with the use of intrapar-

Patients achieving complete cervical dilatation but delivered by cesarean section (%)	*Mean cervical dilatation at cesarean section (cm)*	*Duration from membrane rupture to delivery* (min)*
43	7.8	445
21	6.6	332
28	6.9	329
$p = 0.05$	$p = 0.02$	$p = 0.07$

tum electronic fetal monitoring as compared with those in patients undergoing auscultation. Seitchik and Rao[23] noted that " . . . the increased use of cesarean sections for the treatment of dystocia in the past decade was associated with the use of electronic monitoring of uterine activity." They further noted that therapeutic goals for oxytocin-augmented uterine activity have been recommended but have no experimental basis. Eighteen percent of their patients in normal spontaneous labor, who experienced spontaneous vaginal delivery, averaged more than 250 Montevideo units (MU). In contrast, the currently used definition of adequate labor by the American College of Obstetricians and Gynecologists[25] includes labor patterns generating as little as 200 MU. In the search for reasons behind the escalating cesarean rates for dystocia, Seitchik and Rao[23] asked, "Is it possible that the premonitor clinician at the bedside, who used palpation for evaluation, achieved greater uterine activity with oxytocin in those patients than we are now obtaining with the electronic monitor as our guide?"

In more recent reports Seitchik et al.[26] advocated the low-dose schedule mainly on the basis of their observations regarding the oxytocin treatment of functional dystocia and the time required for individual doses to cause a steady state in the plasma. In their study population they noted a maximum mean dose of oxytocin of 6.9 ± 5.9 and 4.1 ± 3.8 mU (mean ± 1 SD) for cesarean and vaginal delivery patients, respectively; they concluded that failure to achieve vaginal birth was due to disabilities of the patient and not differences in the application of management principles. We disagree, believing that patients experiencing functional dystocia differ in the amount of uterine activity required to achieve vaginal birth just as do women who are in spontaneous normal labor. Therefore the effective oxytocin doses required by some patients with dysfunctional labor are expected to be much higher than those used in the study of Seitchik et al. We see no evidence that higher doses would not have resulted in the need for fewer cesarean sections. The reports of O'Driscoll et al.,[5] the historic relationship between oxytocin doses and cesarean rates, and the data in this report all support that contention. A randomized, controlled trial comparing low- and high-dose oxytocin schedules with successful vaginal delivery as the end point is needed to provide proof of this contention. The low cesarean rate physician group used a much higher maximum mean dose of oxytocin than the high cesarean rate physician group (18.3 vs 11.9 mU). Neonatal outcomes were no different. The doses used by the low cesarean rate physicians are more than double those of Seitchik et al.[26] (6.9 and 4.1 mU for cesarean and vaginal births, respectively, in their oxytocin-treated patients with functional dystocia).

A further reason for the use of an insufficient dosage of oxytocin has been the perception of increased risk to the fetus associated with its use. We believe that the risks to the fetus of traditional oxytocin dose regimens have been exaggerated and perpetuated without a scientific basis, in spite of its safety regarding both newborn and long-term morbidity. One of the concerns emanates from the belief that traditional or high-dose oxytocin may cause excessive uterine activity resulting in decreased uteroplacental blood flow and fetal hypoxia. Reassuringly, Thorp et al.[27] demonstrated similar umbilical cord blood gas values from those newborns whose mothers received high-dose oxytocin augmentation and those who received no oxytocin. Additionally, oxytocin has often been cited as a cause for "brain damage" particularly in litigation cases, in spite of the work of Nelson and Ellenberg[28] demonstrating no overrepresentation of cerebral palsy in association with oxytocin use in labor (0.3%). These collaborative perinatal project data come from deliveries occurring between 1959 and 1966 when the traditional high doses of oxytocin were in vogue. The low-dose schedule currently recommended by The American College of Obstetricians and Gynecologists[25] and the *Physicians' Desk Reference* is an additional deterrent to the use of appropriate oxytocin doses, particularly in view of legal risks.

Do "modern" (low) oxytocin dose regimens generate "modern" (high) cesarean delivery rates? Probably. We conclude that a cesarean rate reduction program for patients with dystocia will be successful if it includes more oxytocin for more patients for longer periods of time and if physicians wait until more advanced cervical dilatation is achieved before administration of oxytocin.

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Editors' note: This manuscript was revised after these discussions were written.

Discussion

DR. DWIGHT P. CRUIKSHANK, Milwaukee, Wisconsin. Drs. DeMott and Sandmire have presented data that suggest that a significant reduction in the primary cesarean rate can be accomplished by the "three mores"—*more* oxytocin to *more* patients for *more* hours. They confirm the clinical impression that many of us have, that the recent trend regarding oxytocin usage is one of the obvious causes of the increased cesarean rate. They rightly point out that medicolegal concerns probably inhibit oxytocin use, particularly in view of the low-dose schedule currently recommended by the *Physicians' Desk Reference.* Therefore let us examine what the PDR says about oxytocin and why.

In the section regarding Syntocinon oxytocin injection USP (Sandoz) the 1991 PDR (pp. 1969-70) states, "The initial dose should be no more than 1-2 mU/minute. The dose may be gradually increased in increments of no more than 1-2 mU/minute until a contraction pattern has been established which is similar to normal labor." No references are given for these statements. The section describing Pitocin oxytocin injection USP (Parke-Davis) in the 1991 PDR (pp. 1687-9) is even more restrictive, stating "The initial dose should be 0.5-1.0 mU/minute. At 30-60 minute intervals the dose should be gradually increased in increments of 1-2 mU/minute until the desired contraction pattern has been established. Rates exceeding 9-10 mU/minute are rarely required at term." Five references are given for these statements, including three articles by Dr. Joseph Seitchik and colleagues and the 1987 *ACOG Technical Bulletin* No. 110. That bulletin lists four references regarding oxytocin, including the three by Seitchik. It appears that the work of Seitchik et al. provides the scientific basis for the current low-dose oxytocin protocols, we should examine their findings in some detail.

Between 1982 and 1984, Seitchik et al. published four articles in the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY entitled, "Oxytocin augmentation of dysfunctional labor (Parts I, II, III, and IV)."[1-4] In these studies they reported that dysfunctional labor in nulliparous women could be effectively treated with initial doses of oxytocin of 1.0 mU/min, increased by 1.0

mU/min at ≥30-minute intervals to a dose of 4.0 mU/min, and then increased by 1.0 mU/min every hour at doses above 4.0 mU/min. They found that doses higher than 6 to 7 mU/min were rarely necessary. They also demonstrated, using this low-dose protocol, that approximately 40 minutes was required for any given dose of oxytocin to reach steady-state plasma levels and a maximal uterine contractile response and that oxytocin infusion rates of 6 mU/min produced plasma oxytocin levels comparable to those found in normal laboring women.

I believe there are three major problems with these data, the most important of which is patient selection. All patients were nulliparous and at term and were eventually delivered vaginally. Patients who eventually were delivered by cesarean section for dystocia were not studied, but the articles imply that they too received low-dose oxytocin. The problem is that those patients may very well represent a subset of women who require much more oxytocin to achieve uterine activity. Similarly, although 6 mU/min produced plasma oxytocin levels comparable to those found in normal laboring women, patients with dystocia are by definition not normal laboring women. Whether because of qualitative or quantitative differences in oxytocin receptors or for other biologic differences, these patients may need more oxytocin. Finally, although low doses of oxytocin may take 40 minutes to achieve steady-state plasma levels, it is likely that higher doses would do so much more quickly. Whereas this was not studied with regard to oxytocin, it is certainly true of magnesium sulfate and several antibiotics.

There is a growing body of data, from both Europe and North America, that high-dose oxytocin as practiced in the active management of labor protocol[5] (initial dose 6.0 mU/min, increased by 6.0 mU/min every 15 minutes to a maximum of 40 mU/min) can result in very low rates of cesarean delivery for dystocia, because, as O'Driscoll et al. said, "The problem of dystocia has been virtually solved . . . ."[5] Thorp et al.[6] have demonstrated the safety of such an approach for the fetus and neonate.

In fairness to The American College of Obstetricians and Gynecologists, there is a new *ACOG Technical Bulletin* regarding induction and augmentation of labor (No. 157, July 1991). This bulletin contains the moderate-dose oxytocin regimen of Hauth et al.[7] (initial dose 1 to 2 mU/min, increased by 1 mU/min every 15 minutes), as well as the low-dose protocol of Seitchik et al. and the PDR, and even acknowledges the high-dose active management approach of O'Driscoll et al., while stating that clinical experience with the latter in the United States is limited.

There are several other problems with what the PDR says about oxytocin, namely, that it " . . . should not be administered in the face of prematurity, borderline cephalopelvic disproportion, previous cesarean section, over-distension of the uterus and grand multiparity," among others. I believe there are no reliable data to support any of these, and in fact there are data to the contrary for some. I propose that the American College of Obstetricians and Gynecologists convene a consensus conference of investigators, clinicians, and pharmaceutical company representatives to rewrite the PDR and package insert information for oxytocin to develop more reasonable guidelines for its use, so that physicians will not feel medicolegal risk for using oxytocin in a rational manner.

I have only two criticisms of DeMott and Sandmire's article. First, their three physician groups (low, medium, and high cesarean rates) are artificial. In their low group the cesarean rates for dystocia vary from 2.7% to 5.0%, a spread of 2.3%. In their medium group (range 6.6% to 8.8%) the spread is 2.2%. In the high group, however (range 9.4% to 16.0%), the spread is 6.6%. I suggest that there really are two groups and one outlier. Physicians 1 to 4 are one group with cesarean rates from 2.7% to 5.0%. Physicians 5 to 10 are another group with rates of 6.6% to 10.0%. Then there is physician 11, with a cesarean rate for dystocia of 16%. This physician also had an 0.8% forceps rate, compared with a mean of the rest of the group of 7% (range 3.4% to 14.6%). I suspect that rearranging their groups in this way would not alter the results, except to make them more striking.

My other criticism regards the term *failed labor* in their title. In this litigious age we should not refer to "failed" anything—whether forceps or external version or labor. Elsewhere in their article they use the term *nonprogressive labor*. I would prefer that we stop using these terms, as well as the ubiquitous "failure to progress," and return to the tried-and-true term *dystocia*. I tell medical students that dystocia is the Latin term for the syndrome of WCO—Won't Come Out. However, now we see that most will "come out," if we use *more* oxytocin in *more* patients for *more* hours.

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Dr. Richard P. Perkins, Omaha, Nebraska. Drs. DeMott and Sandmire have submitted their extended analysis of operative delivery in Green Bay. After subdividing their practitioners by operative rates, they set about to find differences in practice patterns that would explain these differences in patients not greatly different from each other. In an examination of the data presented, the impression gained by the reader is that the differences discussed are not especially striking, although they appear to satisfy the statistician.

The authors suggest that higher doses of oxytocin given to more patients for longer times will reduce the cesarean section rate. We would suggest that this may be true, but objective analysis of each case is required to determine if, with management by the more aggressive plan, another outcome would be expected. The authors suspected, but did not confirm, that the group with higher rates used lower doses of oxytocin. This was shown for induced labors, but augmentation dose means for the high-rate group fell intermediately between those of the low and medium groups. The same can be said for the high group for cervical starting points, frequency of augmentation, first-stage and second-stage lengths, percent of cesarean patients with complete dilatation, and mean cervical dilatation before cesarean section.

I am not certain that the conclusions are pure without eliminating those babies born by cesarean section who were significantly above the mean in weight and size and then seeing if the difference remained. This should be required if the effect of augmentation is said to be paramount. I am also not certain that the numbers here are great enough to demonstrate the true significance of the differences if they exist.

It is in the best traditions of medicine for each local hospital and group of cooperative physicians to analyze medical practice and modify patterns accordingly. It requires agreement on the reasons for the task and a healthy view toward the goals being those of improved care and outcomes. I applaud this effort by these authors.

I submit that if each physician were required to fill out a questionnaire before performing cesarean section for failure to progress and if these questionnaires were analyzed periodically, a better insight would be achieved and fewer cesarean sections would be done. We are looking at this ourselves. Pertinent questions to be answered would be the following:

1. Has the cervix ever changed?
2. Are contractions adequate? Monitored objectively? Frequent and strong?
3. Is the cervix at least 4 cm dilated?
4. Is there molding of the presenting part?
5. Is the presenting part in the pelvis?
6. Is the bladder empty?
7. Has progress been arrested for at least 2 hours?

Failure to progress is indeed the commonest indication for primary cesarean section, but failure of whom or what to progress is the issue in doubt. Is it the patient or the provider?

Fetal monitors have given us the ability, if not the inclination, to be more patient with slow labor progress. With the declining expertise with midpelvic forceps and the increasing use of epidural analgesia, expected increases in cesarean section have been observed and may well persist in spite of the benevolent redefinition of midforceps recently proposed. The Green Bay study also reminds us that it is far easier to describe behavior than to determine motivation.

There are subtle pressures also. It is not so much fun to await delivery any more because it used to represent a great relief to the patient and the beginning of life. It is now a great relief to the provider and the ending of a slow "death," worrying about the possibility of an unexpectedly bad outcome that is unrelated to anything he did but at risk of being attributed to him.

What we are all looking for in any plan is a set of guidelines that meet the following qualifications: (1) They seem reasonable; (2) they are acceptable to most patients and providers; (3) they are achievable in the patient's circumstances; (4) those who propose them will effectively back us up should we get sued for using them.

The latter represents the greatest crisis of confidence in medicine today, and its lack, more than any other phenomenon, makes reasonable people do unreasonable things sometimes. In time of war, battles are rarely fought by those who plan them. The court seems to function as little more than a graduate level vocational retreat for ex-members of high school debating teams.

In Hawaii and other places there is a little lizard called a gecko, which, under attack, will shed its tail in the hope of fooling or satisfying the enemy while the rest of the creature beats a hasty retreat. The tail, curiously, continues to wriggle enticingly for a time until the creature is safely away. My concern is that in this case the tails of the gecko are current intervention rationales, which have been left behind in an era of intense litigative pressure and which are still twitching. Soon, however, we will have forgotten how these and so many other defensive stratagems spawned during this age of judicial theatrics came to be, and the "standard of care" will at length have been established mindlessly even after the disturbed rationale has been forgotten. This leaves us with the empty feeling that American obstetrics, while ostensibly in pursuit of a body of knowledge, may achieve nothing more significant than chasing part of a tail.

Finally, the authors consistently capitalized *cesarean,* as if it were named after someone. As we know, the word comes from the Latin word meaning "to cut." We come to bury the cesarean section, not to praise it. Perhaps we need another term—the "unnecesarean section rate."

Dr. Charles W. Schauberger, La Crosse, Wisconsin. Someone suggested that Friedman's curve is a use-

ful tool for the management of labor. Others suggest it is used too frequently to justify performing a cesarean section too early. How frequently is Friedman's curve used at your hospital and are there differences within the three study groups?

Dr. Richard A. Hartman, St. Louis, Missouri. We have an institution in St. Louis, Barnes Hospital, at which one physician amassed about 4000 deliveries during which the patients received epidural analgesia, and the question I'm going to ask is about epidurals. That physician has been able to show that if an epidural anesthetic is given at a cervical dilatation of <3 cesarean section rates of >35% result. If the epidural anesthetic is given between 3 and 5 cm of cervical dilatation, the cesarean rate is somewhere between 11% and 35%. If dilatation is >5 cm, the cesarean section rate is about 11%.

We have institutions in St. Louis where the cesarean section rate ranges from 10% to 35%, not just for physicians but for the institutions. There are a lot of people who feel that the epidural analgesic altered the cesarean section rate. Ten years ago 15% of women received epidural anesthetic. Now almost 90% do so. I just wonder what effect this would have on the data.

Dr. James R. Scott, Salt Lake City, Utah. I just wanted to raise the issue of what are adequate contractions and how they were defined in this study. Was there a difference in how physicians actually decided to use oxytocin when there was failure to progress?

Dr. Elliot M. Levine, Chicago, Illinois. I'm wondering if the authors mentioned the fear of a lawsuit as perhaps being a stimulant for cesarean delivery. I'm wondering if, in fact, the authors did look into the litigation history of the obstetricians mentioned in this study.

Dr. William E. Houck, St. Louis, Missouri. I just have a follow-up comment on several other statements about invasive contraction monitoring. Was there any difference between the use of this in the high-risk versus the low-risk groups?

And one other issue—you mentioned the timing of delivery. Was there an increased incidence of deliveries about 5 PM?

Dr. James D. Funnell, Oklahoma City, Oklahoma. I would like to raise a different set of issues that has to deal with those of us involved in a training program. In training programs throughout the United States there is great variation in the use of oxytocics and the teaching of forceps deliveries. While these vary from institution to institution, they must be factored in when studies such as this are reviewed.

Dr. John W. Calkins, Kansas City, Kansas. As a kind of correlate to Dr. Funnell's statement, I found interesting the differences in the number of operative vaginal deliveries among the different obstetricians. I wondered if the authors looked at the number of years in practice (or the experience of the physicians) to see if that correlated with this specific factor because clearly our residents don't get the experience that residents did 10 years ago in forceps deliveries.

Dr. Sandmire (Closing). Dr. Perkins mentioned that, concerning some of these practice patterns that we measured, there really wasn't much difference between the high and medium cesarean rate physician groups. I agree with that but would point out the substantial differences in comparing the low group to the high group or the combined high and medium groups. We still believe that the results and conclusions are valid but also agree with Dr. Cruikshank's suggestion that there should be only two physician groups and one outlier.

Dr. Cruikshank expanded on our concerns relative to inadequate oxytocin doses and spurious contraindications to its use. Appropriate usage of oxytocin depends on proper patient selection, as well as adequate dose levels. The patient with false labor or the patient with an unripe cervix who has insufficient indications for labor induction should not receive oxytocin. On the other hand, virtually all patients with vertex presentations who are experiencing nonprogressive labor should receive adequate doses of oxytocin before the physician resorts to cesarean section.[1] We agree completely with Dr. Cruikshank's recommendation for a consensus conference to develop more reasonable guidelines for its use. Our concerns have previously been forwarded to The American College of Obstetricians and Gynecologists.

We agree with Dr. Perkins that defensive medicine decision making, which we all practice to various degrees, is not a satisfactory response for a scientific discipline. In addition, it usually is not effective. As Dr. Mortimer Rosen has said, "If you have a bad outcome, you're going to be sued. If you have a good outcome, you're not going to be sued. So if you have a bad outcome, you're going to get sued anyway. You might as well have the satisfaction of practicing good medicine."[2] Furthermore, even by practicing defensive medicine, you're not going to avoid the lawsuit. This emanates from the creativity of the experts who will be able to testify that some management decision you made was questionable. This will be presented to the insurance company and the insurance company will settle the case if the demand is low enough. In Wisconsin we have "frequent flyers" from both coasts who come in and testify against Wisconsin obstetricians, and they have a level of creativity that is very high. I hate to paint a bleak picture, but I think Dr. Rosen is right. We have to guard against the decisions that are based on defensive medicine.

Dr. Schauberger asked about the Friedman curve. We didn't test that. That's something that the individual nurse decides. It's done in most but not all cases.

We generally agree with Dr. Perkins' list of questions to ask ourselves before performing a cesarean section for failure to progress. Perhaps to expand slightly on his assessment of the contractions, we offer our definition of adequate contractions not as how hard the

uterus feels, how frequent the contractions are coming, how long they last, or what the bottom line on the monitor strip demonstrates. We define adequate contractions as those that produce progressive dilatation and/or descent; and if they are not doing that, we do not have adequate contractions. Admittedly, with the presently available interventions, we are unable to achieve adequate contractions in some patients.

Regarding epidural anesthetic—I think that to give a patient something that will interfere with her ability to push and maybe the contraction strength is the last thing that I want to do. We don't use epidural anesthetics in Green Bay. I think that there are many studies, although not randomized, that show that epidural anesthesia increases the cesarean rate.

I answered Dr. Scott on what is the definition of an adequate contraction. Dr. Levine asked about the litigation histories of the 11 obstetricians. As I said 2 years ago, regarding these 11 obstetricians, they all know about the Green Bay lawsuits because some of their partners have been sued, and for the young ones who haven't, I tell them, "Be patient." So they all have equal knowledge of the liability risks.

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Vaginal birth after cesarean delivery: Are there useful and valid predictors of success or failure?

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Jackson, Mississippi

OBJECTIVE: Before parturition are there useful and valid predictors of successful or unsuccessful vaginal birth after previous cesarean birth that could be used to enhance the obstetric care of a patient and her pregnancy?

STUDY DESIGN: The clinical course and outcome of all patients who attempted vaginal birth after cesarean delivery at one level III center during 1989 were evaluated to identify factors prognostic of a successful or unsuccessful patient group: use of this information in stepwise logistic regression and cluster analysis was disappointing.

RESULTS: No single criterion or optimal clusters of factors were found and no equation achieved >75% predictability of outcome with acceptable sensitivity and specificity.

CONCLUSIONS: Before parturition prediction of outcome of vaginal birth after cesarean delivery is tenuous regardless of past obstetric history or recent clinical parameters. Thus it seems appropriate to encourage a trial of labor in almost all patients with a prior low-segment uterine incision (transverse or vertical) unless there is a strong physician or patient-derived contraindication to such an undertaking. (AM J OBSTET GYNECOL 1992;166:1811-9.)

Key words: Vaginal birth after cesarean delivery

Since the early 1970s in the United States there has been a trend away from the practice of elective repeat cesarean delivery toward trial of labor in selected patients who have undergone abdominal delivery.[1, 2] Abundant obstetric literature has substantiated the safety and efficacy of this evolution in obstetric care.[3-6] The trend has been aided significantly by the American College of Obstetricians and Gynecologists, which in published guidelines clearly support and encourage the concept of vaginal birth after cesarean delivery.[7]

In spite of knowledge regarding the safety and success rate of vaginal birth after cesarean delivery, a method to reliably predict a successful or unsuccessful attempt before the onset of labor would further enhance clinical care. The present investigation was undertaken to determine if there is an association between specific obstetric variables or combinations of these variables and a successful or unsuccessful trial of labor on an individual basis.

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Material and methods

A computer-directed search of the medical records of obstetric patients who delivered at the University of Mississippi Medical Center during the calendar year 1989 was undertaken to identify all women with a previous cesarean delivery who underwent a trial of labor in a subsequent pregnancy. The records of 312 patients were identified for analysis. Twenty-four charts could not be obtained. Thus 93% of all possible patients were available for study, and these subjects were divided into women who experienced a vaginal birth (successful) and those who had an abdominal delivery after a trial of labor (unsuccessful). Nineteen specific obstetric variables or factors (Table I) for each patient were obtained from the clinical courses and outcomes of both successful and unsuccessful attempts at vaginal birth after cesarean delivery.

Cervical assessment (favorability) was performed by recording cervical dilatation, effacement, and station and using this information for a modified Bishop score with a maximum score of 6. The terms recurrent and nonrecurrent indications for previous cesarean delivery are defined as follows: recurrent—failure to progress, cephalopelvic disproportion; nonrecurrent—fetal distress, twins, breech, severe preeclampsia, herpes, or prolapsed umbilical cord.

Appropriate univariate analyses were conducted with t tests or χ^2 analysis. Stepwise logistic regression with probablity of entering the model equal to 0.05 was used

Table I. Factors considered in study of vaginal birth after cesarean delivery study

Variable	*No.*
Race	309
Age	309
Height	254
Weight	299
Gravidity	312
Parity	312
Cervical dilatation in centimeters	309*
Effacement	299*
Station	301*
Modified Bishop score	309*
No. of previous cesarean sections	312*
No. of previous vaginal deliveries	312*
Indication for previous cesarean section	312*
Estimated gestational age	309*
Spontaneous rupture of membranes	312
Placental grade by sonogram	85
Fluid status by sonogram	97
Spontaneous uterine activity	312*
Estimated fetal weight by sonogram	101*

All 19 factors investigated for relationship and prediction validity to successful vaginal birth after cesarean delivery are listed with those identified by asterisks if significance ($p < 0.05$) was observed.

*Significant at $p = 0.05$ level.

Table II. Logistic regression summary

Variable	*Coefficients*	
	Equation 1	*Equation 2*
Constant	−4.4183	−8.6165
Estimated fetal weight by sonogram	0.0010	
No. of previous cesarean sections	0.7719	0.8326
Cervical dilatation in centimeters		−0.4803
Estimated gestational age		0.2160
No. of patients	101	306
Sensitivity (%)	60.4	38.4
Specificity (%)	66.0	87.9
Correct (%)	63.4	71.9

Two different equations with stepwise logistic regression procedure produced different outcomes.

to determine if the number of these variables could be reduced and combined to explain the probability of vaginal birth, p, given by an equation of the form

$$p = 1/(1 + \exp^{-x})$$

where x is a regression equation involving the selected variable and their coefficients. Cluster analysis was performed to detect natural groupings of the independent variables regardless of the dependent variable. The Statistical Analysis System (Statistical Analysis Systems, Inc., Cary, N.C.) was used for all analyses. A significant difference was defined as a p value of ≤ 0.05.

Table III. Cumulative frequency distribution of p values derived from either two equations with associated predictive values

	Equation 1		*Equation 2*	
	Vaginal	*Cesarean*	*Vaginal*	*Cesarean*
$p = 0.050$	5.7	0.0	9.7	1.0
$p = 0.100$	11.3	0.0	18.8	1.0
$p = 0.150$	20.8	0.0	30.4	3.0
$p = 0.200$	24.5	0.0	45.4	10.1
$p = 0.250$	24.5	2.1	55.6	14.1
$p = 0.300$	28.3	4.2	69.6	29.3
$p = 0.350$	34.0	10.4	72.9	42.4
$p = 0.400$	49.1	16.7	76.8	48.5
$p = 0.450$	56.6	29.2	82.1	56.6
$p = 0.500$	66.0	39.6	87.9	61.6
$p = 0.550$	71.7	56.2	92.3	66.7
$p = 0.600$	86.8	60.4	96.1	74.7
$p = 0.650$	90.6	66.7	97.1	77.8
$p = 0.700$	96.2	79.2	98.6	81.8
$p = 0.750$	96.2	85.4	98.6	89.9
$p = 0.800$	98.1	91.7	99.0	92.9
$p = 0.850$	100.0	97.9	100.0	97.0
$p = 0.900$	100.0	100.0	100.0	98.0
$p = 0.950$	100.0	100.0	100.0	99.0
$p = 1.000$	100.0	100.0	100.0	100.0

Results

Among the 4211 pregnant patients delivered of live-born infants at the University of Mississippi Medical Center during 1989, there were 495 women (11.7%) who had prior abdominal delivery. A trial of labor with attempted vaginal delivery was undertaken in 336 patients (67.9%). There were 24 with indeterminant outcomes due to lost charts (7.1%), 212 (63.1%) successful outcomes, and 100 (29.8%) who required repeat abdominal delivery.

Table I lists the 19 factors that were studied and the number of deliveries for which data in each case was available for analysis. Ten factors individually were significantly related to birth outcome ($p < 0.05$). Vaginal birth occurred more often than required repeat cesarean delivery in the presence of spontaneous labor, a favorable cervical vaginal examination at the time of hospital admission, lower ultrasonographically estimated fetal weight (with biparietal diameter and abdominal circumference) and gestational age, and previous vaginal delivery with fewer prior cesarean births, as well as when prior abdominal delivery was associated with nonrecurrent indications (Fig. 1).

Combinations of two or more factors for prediction of outcome was undertaken. Two very different outcomes were found with the stepwise logistic regression procedure. The reason for this difference is that the estimated fetal weight by ultrasonography was measured in only 101 of the subjects, and logistic regression permits subject inclusion only when data for all dependent and independent variables are present for the

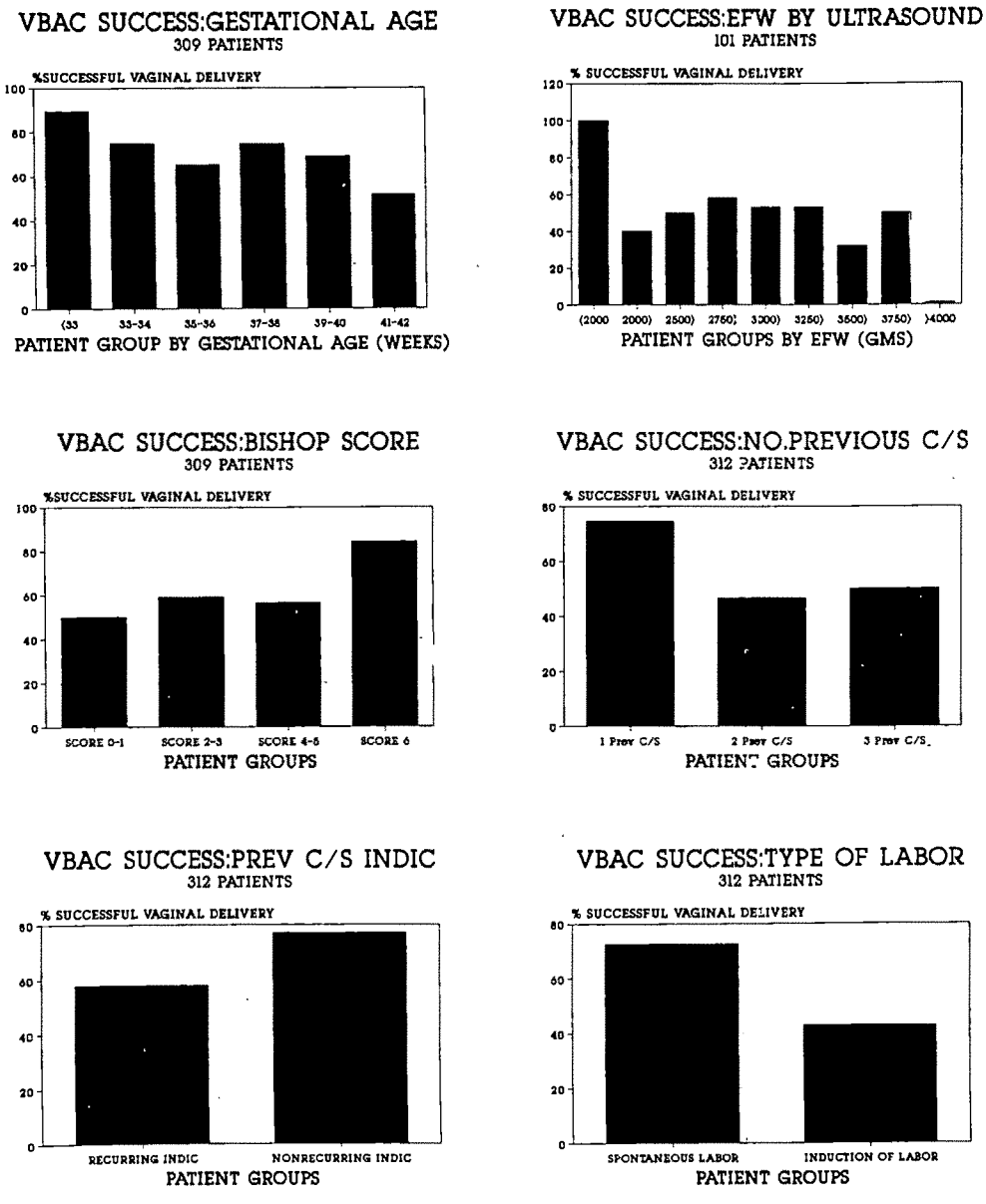


Fig. 1. Six of 10 factors significantly related to birth outcome and vaginal birth after cesarean delivery *(VBAC)* are depicted graphically in this display.

computation of estimates. These two equations are summarized in Table II.

In the first equation which includes the estimated fetal weight, it is apparent that the sensitivity (the probability of correctly predicting a cesarean birth, $p > 0.500$) is 60.4% and the specificity (the probability of correctly predicting a vaginal birth, $p < 0.500$) is 66.0% and that the overall rate of outcome prediction is 63.4%. In the second equation estimated fetal weight was dropped from the list of independent variables. Thus data from 306 of the study subjects were used to compute an estimate that produced an equation that has a lower sensitivity (38.4%), a higher specificity (87.9%), and a better rate of overall prediction (71.9%) than the first equation.

Further comparison of the two equations can be expressed by the respective values of R^2, which is the proportion of log likelihood explained by the equation. For equations 1 and 2 these are 0.1018 and 0.1552, respectively, indicating that equation 2 is slightly better than equation 1. However, neither of these figures indicates a clearly superior fitting equation.

Cumulative frequency distributions of the p value given in Table III provide a final possible meaningful use of these equations. For equation 1, $p \leq 0.300$ would predict correctly 28.3% of the vaginal births and in-

correctly 4.2% of the cesarean births, and $p \geq 0.700$ would correctly predict 20.8% of the cesarean births and incorrectly predict 3.8% of the vaginal births. This leaves a majority, 67.9% of the vaginal births and 75% of the cesarean births, that give similar values of p for the two very different outcomes. Similar cutoff values of 0.150 and 0.700 for equation 2 leave 68.2% of the vaginal births and 78.8% of the cesarean births giving similar values of p. These gray areas are indicative of the general lack-of-fit of both statistical models.

The lack of good separation in our equations for outcome prediction is confirmed by the results of cluster analysis. On the basis of the patterns of the pseudo F statistic and the cubic clustering criterion, it is concluded that no optimal clusters of clinical criteria (Table I) were found.

Comment

Women with a previous abdominal delivery who attempt a subsequent vaginal delivery have a high chance of success regardless of clinical parameters. Overall success rates at various institutions in the United States range between 50% and 80%.[3, 5-9] At the University of Mississippi Medical Center, the successful rate of vaginal birth after cesarean delivery in 1989 was approximately 63%, almost identical to the 62% rate reported in a collaborative project from this institution and the University of Alabama undertaken in 1981.[10]

A strong relationship between single or clusters of specific obstetric variables and subsequent mode of delivery (vaginal or abdominal) in patients with previous cesarean operations was not observed in this investigation. In contrast, Silver and Gibbs[11] found that previous vaginal delivery, a nonrecurrent indication for prior cesarean delivery, and any cervical dilatation during the first 2 hours of labor augmentation to be significant global predictors of successful trial of labor/vaginal birth after cesarean delivery in a prospective analysis of 98 patients with one previous abdominal delivery.

Whiteside et al.,[12] in a retrospective review of 194 patients, observed that several specific obstetric variables were associated with successful vaginal birth after cesarean delivery. These included a nonrecurrent indication for previous cesarean birth, a previous vaginal delivery, a duration of labor <24 hours before primary cesarean section, a fetal birth weight <4000 gm, and cervical dilatation >4 cm at the time of admission for trial of labor. In these studies[11, 12] each variable was examined independently and cluster analysis was not undertaken.

Recently, Rosen and Dickinson[8] reported a meta-analysis of 29 studies of vaginal birth after cesarean delivery that investigated the association between successful trial of labor and various individual preexisting conditions. With data from 8770 patients these investigators concluded that a trial of labor, regardless of preexisting obstetric variables, is associated with a >50% chance of successful vaginal birth after cesarean delivery. Compatible with the present findings, no cluster analysis from this large body of patients would be expected to render predictive accuracies for success or failure on an individual basis that would approach a clinically useful 80% to 100% range.

The use of a fetal-pelvic index has been investigated to evaluate its efficacy for the prediction of failure of vaginal birth after cesarean delivery.[13] Using the concept of the fetal-pelvic index to compare the fetal head and abdominal circumferences by ultrasonographic mensuration with the respective maternal pelvic inlet and midpelvic circumferences by radiogaphic pelvimetry, Thurnau et al.[13] studied 65 patients admitted for trial of labor on vaginal birth after cesarean delivery. All 13 patients with a positive fetal-pelvic index value failed to progress in labor and a cesarean delivery was required (positive predictability 1.00). In contrast, 47 of the 52 patients with a negative fetal-pelvic index value delivered vaginally (negative predictability 0.90). Of the five with false-negative fetal-pelvic index values, four fetuses persisted in an occipitoposterior presentation and failed to progress in labor. The investigators observed that neither pelvimetry nor ultrasonography alone provided accurate identification of fetal-pelvic disproportion. Although somewhat cumbersome and demanding of special skills, it is possible that use of this clinical approach late in gestation or early in labor might avert long unsuccessful labor trials in those pregnant women destined to fail attempted vaginal birth after cesarean delivery.

The pilot study to our present investigation examined the results from a sample group of only 100 patients (50 patients who had successful and 50 patients who had failed vaginal birth after cesarean delivery) from the larger patient population reported in this study. Strong associations between pairs of specific obstetric variables and outcome (successful or failed vaginal birth after cesarean delivery) were found. The strongest pairing of variables were associated with a >70% successful vaginal birth after cesarean delivery rate or with a <30% successful rate. Patients especially prone to a failed trial of labor were those with a gestational age >40 weeks, a recurrent indication for prior cesarean delivery, an unripe cervix, and initiation of labor by induction (or any paired combination of these variables).

Expansion of the pilot study population to include all patients who attempted a trial of labor during 1989 at University of Mississippi Medical Center was under-

taken to further validate or refute these initial findings. Additionally, it was thought that subsetting of several variables made possible with a larger study group might show even stronger associations with successful or failed vaginal birth after cesarean delivery that would be of clinical value. Unfortunately, the reverse situation occurred.

Regardless of the analytic equation (1 or 2) used, the optimal subset of variables (Table II) produced overall prediction rates of only 63% and 71%, respectively. In our patient population and obstetric practice the success rate of vaginal birth after cesarean delivery is 63%, whereas in the nation at large the success ranges between 50% and 80%. Thus all the statistical methods used in this investigation for individual patients and that of meta-analysis for groups of patients do not provide better methods of prediction than the general overall expected rate of success in our population. Thus there appears at present to be no specific variables or combinations of historic or clinical variables that would allow the clinician to accurately identify and predict a successful or failed vaginal birth after cesarean delivery on an individual basis.

On the basis of well-documented minimal maternal and fetal morbidity associated with a trial of labor as reported by meta-analysis of a large patient experience in 29 investigations, it seems appropriate to encourage a trial of labor *in almost all* patients with prior low-segment incisions (transverse or vertical) regardless of their obstetric history or their recent clinical parameters. Exceptions to this policy would be the infrequent, carefully selected patient deemed to be at too great a risk for trial of labor on the basis of medical and/or obstetric considerations(s).

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Discussion

DR. SUZANNE TRUPIN, Chicago, Illinois. Obstetricians encourage vaginal birth after cesarean delivery because this straightforward course of management does more to improve the safety and economics of birth while enhancing the birth experience than any other single intervention. Dr. Pickhardt and his colleagues from Jackson, Mississippi, in their retrospective study analyzed 312 patients, representing 93% of the trial of labor in his institution during 1989. Their hope was to establish criteria that would allow pregnant women to avoid lengthy, unsuccessful labor trials. It is an interesting concept and one that has not been firmly established for any category of parturient—induction, postdates, presumed macrosomic fetus, or diabetic pregnant woman. Although the investigators found no single variable nor any constellation of variables predictive of success, the data indicates categories of pregnant women especially prone to failure by trend analysis. Specific cases included those of gestational age >40 weeks, those with the recurrent indications of cephalopelvic disproportion and failure to progress, those with an unripe cervix as defined by a modified Bishop's score, and those undergoing labor induction. This paper facilitates the health promotion issue of trial of labor as it contributes to the growing body of literature encouraging us to defocus the issue from who should not undergo vaginal birth after cesarean delivery by demonstrating that patients can succeed regardless of past obstetric history or recent clinical parameters.

Medical parameters of safety have progressively broadened indications for vaginal birth after cesarean delivery. This fact, coupled with the support of the American College of Obstetricians and Gynecologists, has allowed for the expansion of this service nationwide. Yet only 37% of patients with previous cesarean births did not have a trial of labor. This is significantly improved over the elective repeat cesarean rates of 98% reported in 1979 and 92% in 1984.[1] However, this rate is far short of the goal of 11% elective repeat caesarean which some have set their sights on.[2] The economic savings of vaginal birth after cesarean delivery and consumer demand are further stimuli to reaching this goal, yet studies indicate that physician bias is still an im-

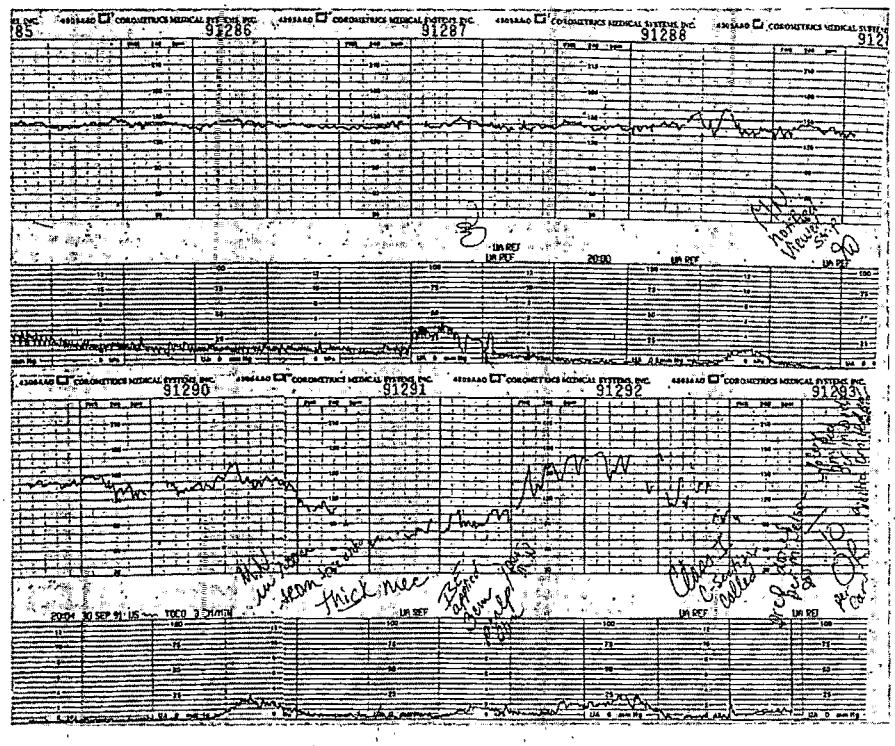


Fig. 1. Uterine tracing of 24-year-old woman, gravida 2, para 1, undergoing trial of labor.

portant influence on individual cesarean birth rates.[3] All of us are influenced by a host of less definable factors such as these two recent cases in my institution: a 24-year-old woman, gravida 2, para 1, undergoing a trial of labor (Fig. 1) or the more frightening 31-year-old woman, gravida 4, para 2, with a previous cesarean section (Fig. 2), both of whom were found to have complete uterine scar dehiscence and massive intra-abdominal bleeding at the time of operative intervention.

My questions regarding this article are (1) What are your criteria for candidacy for vaginal birth after cesarean delivery? Of the 183 patients who did not undergo a trial of labor, what percentage would have been candidates on medical criteria alone? It is possible that because 37% of the population available for study were not studied, the statistics for so-called recurrent indications would indeed be more revealing. (2) Were the authors able to scrutinize the indications for the original and the repeat cesarean birth? Were patients undergoing a trial of labor more quickly assigned the diagnosis of cephalopelvic disproportion than during their first birth? How often was trial of labor abandoned due to the patient withdrawing her consent? (3) Should the labels recurrent and nonrecurrent be discouraged or abandoned? and (4) Because the use of a modified Bishop's score has never been validated, do the authors anticipate that the inclusion of the criteria of cervical position and consistency will enhance predictability?

This article, and the others addressing the issue of successful vaginal birth after cesarean delivery, should further enhance the concept of trial of labor for all. The facts indicate that most patients will succeed. I further propose three ways that we as obstetricians can encourage vaginal birth after cesarean delivery. First, we do not ask our patients, "Are you going to have a vaginal birth?," but rather, "Are you going to have a trial of labor, for if you do you are likely to have a vaginal birth!" Second, we make counseling for trial of labor part of routine prenatal care in spite of the fact that it is not listed as a health promotion objective in the Public Health Service 1989 document on the content of prenatal care.[4] Counseling for vaginal birth after cesarean delivery ideally should begin immediately in the postpartum care of the primary cesarean patient. Third, we do not schedule elective repeat cesarean births. This will discourage us from allowing our convenience to deprive patients of their opportunity to succeed. I look toward the future where elective repeat cesarean birth will be as rare as elective primary cesarean.

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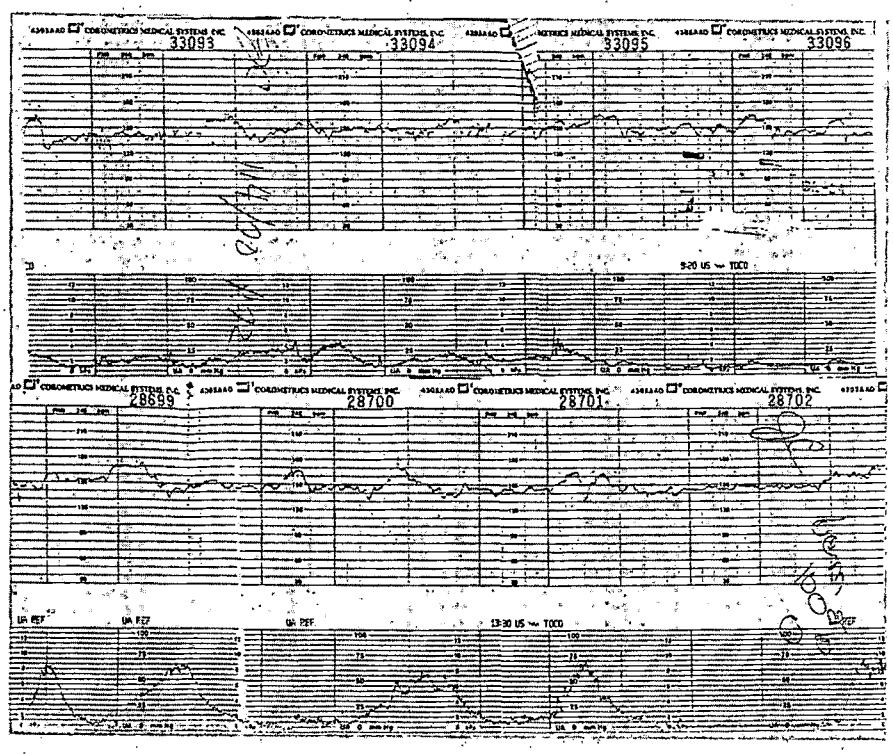


Fig. 2. Uterine tracing of 31-year-old woman, gravida 4, para 2, with one previous cesarean section, undergoing trial of labor.

Table I. National Maternity Hospital, Dublin, uncorrected perinatal mortality data

Year	*Overall perinatal mortality rate*	*Perinatal mortality rate in cases scheduled for vaginal birth after cesarean delivery*	*Perinatal losses directly attributable to vaginal birth after cesarean delivery*
1983	21.0/1000	24.8/1000	2 of 5
1984	15.2/1000	28.2/1000	1 of 7
1985	16.1/1000	20.2/1000	4 of 5
1986	15.4/1000	37.0/1000	3 of 6
1987	14.7/1000	23.0/1000	2 of 4
1988	13.3/1000	23.5/1000	1 of 4
1989	14.9/1000	11.7/1000	None of 2
TOTAL	15.8/1000	24.0/1000	13 of 33

4. Caring for our future: the content of prenatal care. A report of the Public Health Service expert panel on the content of prenatal care. Washington: Public Health Service, Department of Health and Human Services, 1989.

Dr. Leslie Iffy, Newark, New Jersey. The study of Dr. Pickhardt and his associates rests on certain assumptions that to the authors appear self-evident.

The first premise is that the "obstetric literature has substantiated the safety and efficacy" of vaginal birth after cesarean delivery. In support of this conclusion, the writers quote five references. Having experienced no difficulty in identifying another five papers that had found substantial risks involved with the indiscriminate use of vaginal birth after cesarean delivery,[1-5] I am compelled to consider the authors' choices subjective. Of particular interest is the study of Meeham et al.[1] who found a 30.3 per 1000 perinatal mortality rate in association with vaginal birth after cesarean delivery as compared with 22.5 per 1000 in their overall obstetric population and 10.6 per 1000 among patients delivered by repeat cesarean section. Interestingly, the yearly Clinical Reports of the National Maternity Hospital, Dublin,[6] the bastion for vaginal delivery, show that between 1983 and 1989 the perinatal mortality rate in association with vaginal birth after cesarean delivery trials was 50% higher than in the overall institutional population (Table I). A close evaluation of the relevant data reveals that the surplus derived from losses directly attributable to attempted vaginal birth after cesarean delivery.

The second assumption of the authors is that because

of the safety of vaginal birth after cesarean delivery it is "appropriate to encourage trial of labor . . . regardless of past obstetric history or recent clinical parameters." Actually, even those reassuring data that the writers quoted rested on selective use of vaginal birth after cesarean delivery, undertaken after the elimination of risky cases. Thus the relative safety in well-selected cases does not, by any means, permit the conclusion that it is still safe even if the risk factors are ignored.

The third cornerstone for the conclusions of the authors is the assumption that it is a >75% predictability of failure of vaginal birth after cesarean delivery that offers an "acceptable sensitivity and specificity" for an elective cesarean section. For this arbitrary stipulation they offer no explanation. It is understood that the choice between alternative procedures depends on respective risk-benefit ratios. Noting that the maternal infectious morbidity after a cesarean section performed after active labor is about three times as high as after an elective operation,[7] it seems to me that a less than average (i.e., <65% to 70%) chance for vaginal delivery probably serves as a reasonable justification for an elective repeat cesarean section. By its very nature, labor and delivery has an intrinsic fetal risk, as demonstrated by Hellman and Prystowsky[8] decades ago. That this risk still exists is indicated by the earlier quoted data showing that vaginal birth after cesarean delivery carries a higher rate of perinatal mortality than does elective cesarean section. The same conclusion derives from the recently conceded inability of fetal heart rate monitoring to accurately predict neonatal outcome.

In their review of the literature, the authors mention one study[9] that found excellent predictive value for vaginal birth after cesarean delivery failure on the basis of the fetopelvic index. Indeed, the fetopelvic relationship has been shown to be a good predictor for successful vaginal delivery.[10] I find it unfortunate therefore that the authors elected to draw far reaching conclusions about the unpredictability of failed vaginal birth after cesarean delivery without incorporating this parameter of investigation into their study. Their suggestion, namely, that the limited predictability of the outcome warrants indiscriminate use of vaginal birth after cesarean delivery in clinical practice is more likely to discredit this approach in the long run than to promote its use under appropriate circumstances.

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Dr. Marvin L Hage, Durham, North Carolina. I would first question the implications of your findings by your exclusion of patients in the study from the analysis. In other words, the exclusions may have biased your interpretation of the significance of the results. Second, what information was provided to the patients? Would their behavior have been altered related to undertaking a trial of labor with these kind of predictive values?

Dr. Martin (Closing). First of all, Dr. Trupin asked why 180 patients did not undergo trial of labor in our study. The criteria to exclude these patients were multiple and varied. We are fairly liberal in Jackson to undertake vaginal birth attempts after prior cesarean operations. We attempt, as you noticed, more than 60% of possible candidates. Even Dr. Bruce Flamm, who is a very great proponent of vaginal birth after cesarean delivery, reported only a 37% or 38% rate of trial labor at his institution. In addition to patients with classic uterine scars or fetal malpresentations, we exclude women who have medical complications of pregnancy that we judge shouldn't warrant an attempt, patients with marginal pelvimetry, and a variety of special situations that are difficult to quantify. The use of medical criteria alone would include complicated hypertension, poorly controlled diabetes mellitus, and so forth. Generally, a patient with a prior low vertical incision is encouraged to undertake a trial of labor.

The issue of a failed trial of labor in association with a failure to progress was raised by Dr. Trupin. We did not do a definitive analysis of why each of the 100 women who underwent unsuccessful trial of labor didn't succeed. The purpose of this investigation was to see if we could develop a type of Bishop score for the individual patient to predict the possibility of successful vaginal birth after cesarean delivery. Our purpose was not to argue the issue of whether trial of labor after cesarean is safe or unsafe as raised by Dr. Iffy.

Dr. Roy Pitkin's comments in a recent *Obstetrics and Gynecology* commentary after an article by Dr. Jim Scott are relevant at this point in that vaginal birth after cesarean delivery has been shown to be safe in capable hands, but this is not a warrant for unlimited use of it without appropriate restraints and care.

Should the terms *recurrent* and *nonrecurrent* be abandoned? I don't know. We use them because they're fairly traditional terms and so that others will understand what we are talking about.

At the University of Mississippi Medical Center we also offer a trial of labor to all patients with acceptable criteria as if it were the expected norm. This is in answer partially to Dr. Hage's questions. A lot of old issues die slowly, and it will take some time to educate physicians and patients to this general policy of widespread trial of labor. As Dr. Jerry Joseph indicated last year, how the physician presents this issue to the patient from the beginning of the next pregnancy has tremendous importance.

In recent years at Mississippi we have educated our resident staff to expect that vaginal birth after cesarean delivery will be undertaken as a rule rather than an exception.

Finally, does favorable cervical condition enhance the predictability of a successful trial of labor? During the first half of this study we evaluated a subset of this patient population. At that early stage we were of the impression that a favorable Bishop score was strongly associated with success or the converse. The data seemed to suggest that if a patient got to 41 to 42 weeks, had an unfavorable cervix, and the fetal vertex was not engaged, that the chance of a successful trial of labor was almost nil.

It turned out that as the numbers expanded to a full population, the association between favorable Bishop score and vaginal delivery was not as strong as anticipated from early data. I suspect that if we could increase this population to a thousand patients, we would see again that our preconceived biases about who will succeed and who won't will fall by the wayside if we consider the individual patient before the onset of labor.

Whiteside's group is the only one that I can recall that has evaluated conduct of labor as a predictor of vaginal delivery. I think this area would be an appropriate place to study next.

Dr. Iffy asked about the risk of vaginal birth after cesarean delivery. As I've indicated, I don't really think that this is an appropriate podium on which to debate that issue. The Dublin information, by the way, did not exclude perinates that had congenital abnormalities and so forth; and of course, their uncorrected perinatal mortality rates were high. When that correction is made, I don't think the risk to the perinate was significantly increased above controls.

Indiscriminate use of vaginal birth after cesarean delivery was addressed earlier.

In closing, we should emphasize again that trial of labor after previous cesarean birth is a procedure that works well. It does require, however, that each of us as responsible clinicians enter into it with caution and that patients are monitored very closely, as we would monitor any patient in labor. Three of the women that had rupture of transverse incisions in Dr. Jim Scott's recent report in *Obstetrics and Gynecology* were not being monitored closely at the time. We need not have that happen. It appears that we cannot predict the success or failure of the individual patient any better than overall chance would suggest.

Comparison of dynamic image and pulsed Doppler ultrasonography for the diagnosis of the small-for-gestational-age fetus

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New Orleans, Louisiana

OBJECTIVE: Because poor fetal growth is a significant cause of perinatal morbidity and mortality, a prospective study was undertaken to evaluate the ability of real-time ultrasonography and Doppler velocimetry to detect the small-for-gestational-age fetus.
STUDY DESIGN: A prospective study of 136 women at risk for fetal growth abnormalities was conducted. Patients were delivered within 3 weeks and had live-born, nonanomalous, singleton infants. The relative estimated fetal weight (estimated fetal weight divided by the median birth weight for gestational age) and the systolic/diastolic ratio were measured and compared with receiver-operator characteristic curves. In this method the area under the curve is the index of performance.
RESULTS: Forty-six infants were small for gestational age. Although both relative estimated fetal weight (area under the curve = 0.923) and systolic/diastolic ratio (area under the curve = 0.837) were significantly associated with the small for gestational age fetus, the former was more strongly correlated, $p = 0.021$.
CONCLUSION: Relative estimated weight is more sensitive and specific and should be the preferred parameter when gestational age is known. (Am J Obstet Gynecol 1992;166:1820-6.)

Key words: Ultrasonography, Doppler velocimetry, small-for-gestational-age fetus

Management of the small-for-gestational-age (SGA) fetus requires accurate antenatal identification. To date prenatal diagnosis has revolved around a group of ultrasonographic surveillance methods. Traditional biometry, assessing fetal size, and Doppler velocimetry of the umbilical artery have been evaluated. Both methods have a sensitivity of nearly 80%.[1, 2] Previous studies have found velocimetry to have good correlation with neonatal outcome.[1, 3, 4] Standard ultrasonography was more sensitive, but statistical comparison of techniques was not offered.[1, 5]

The intent of this study is to compare dynamic image and pulsed Doppler ultrasonography for the antenatal identification of the SGA fetus. Specifically, the utility of the velocimetric systolic/diastolic ratio of the umbilical artery and estimated fetal weight relative to gestational age were compared with receiver-operator characteristic curves and standard 2 × 2 tables for dichotomous variables. Relative estimated fetal weight was selected for comparison because of higher sensitivity than other standard ultrasonographic parameters.[5, 6]

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Material and methods

The study population comprised 136 patients with singleton pregnancies whose infants were born alive within 3 weeks of ultrasonography. Patients were primarily private and were racially mixed. Only women with a known last menstrual period, intact membranes, and confirmatory early physical examination and ultrasonogram were included. All study patients had live-born infants that were not anomalous. The biparietal diameter, abdominal circumference, and femur length were recorded, as previously described.[6] Fetal weight estimation was calculated by the following formula, which is known to fit our patient population[7]:

$$EFW = 10^{1.3598 + 0.051\,AC + 0.1844\,FL - 0.0037\,AC \times FL}$$

where EFW is estimated fetal weight, AC is abdominal circumference, and FL is femur length. Relative weight was then calculated by dividing the estimated fetal weight by the median birth weight for the gestational age at which the study was done, the latter being taken from standard tables for sea level.[8] For identification of an SGA fetus, a relative weight of 0.80 was selected, because the 10th percentile boundary is approximately 80% of the median birth weight.

Fetal umbilical artery velocity waveforms were measured with a pulsed, gated image—directed ultrasonography system. A free-floating portion of the umbilical cord was identified with dynamic image ultrasonography. The Doppler was then initiated, with the gate placed on the umbilical artery. The typical wave-

Table I. Patient demography

	SGA (n = 46)	*Non-SGA (n = 90)*	*Significance*
Menstrual age at delivery (days)	261.2 ± 23.0	274.4 ± 13.1	$p = 0.001$
Menstrual age at study (days)	254.7 ± 23.2	267.4 ± 14.0	$p = 0.001$
Interval between study and delivery (days)	6.5 ± 6.3	7.0 ± 6.0	NS
Birth weight (gm)	1946 ± 653	3372 ± 689	$p = 0.001$

Data expressed as mean ± SD. *NS*, Not significant.

form was identified by aural and visual evaluation.[9] Fetuses were analyzed during nonbreathing periods. Maximal systolic and minimal diastolic velocities were measured. A systolic/diastolic ratio was calculated from three or more representative waveforms and the results averaged. For the purpose of this study, a systolic/diastolic ratio of ≥3.0 was considered abnormal.[1, 9]

Infants were weighed after delivery. An SGA infant was one who weighed <10th percentile for the gestational age at delivery. Weight tables specific for sea level were used.[8]

To evaluate the accuracy of ultrasonographically obtained parameters in the detection of the SGA newborn, we plotted receiver operating characteristic curves (sensitivity versus 1-specificity). The area under the curve for both relative weight and systolic/diastolic ratio and its standard error were calculated by the method of Hanley and McNeil.[10] Differences in the ability to predict SGA between the two methods were determined by comparing the area under the curve for each curve.[11]

Correlation of dichotomous variables was by χ^2 or Fisher's exact test. Group means were compared by Wilcoxon rank sums. Statistical significance was accepted at 0.05.

Results

Of 136 study participants, 46 (33.8%) delivered SGA newborns. Information concerning gestational age and birth weight is presented in Table I. In 88 (64.7%) of patients studied, the interval between study and delivery was ≤7 days. No difference in this interval was evident between the two groups. SGA newborns were smaller and were delivered earlier.

The receiver operating characteristic curves for relative weight and the Doppler-derived systolic/diastolic ratio are presented in Fig. 1. The areas under the curve and their standard error were 0.923 ± 0.028 for relative weight and 0.837 ± 0.040 for the systolic/diastolic ratio. The curve for relative weight was significantly better than for the systolic/diastolic ratio, $Z = 2.04$, $p = 0.021$.

Presentation of selected points along the receiver-operator characteristic curve for relative weight and systolic/diastolic ratio are presented in Tables II and III. Table II presents data when arbitrary boundaries are selected, and Table III illustrates the patient distribution when the point on the curve is selected to be closest to 100% sensitivity and 0% false positive. Both relative weight and systolic/diastolic ratio are strongly associated with SGA, $p < 0.001$. The predictive value of the positive and specificity for relative weight are about 20% greater than those of the systolic/diastolic ratio.

Comment

Significant differences exist between SGA and non-SGA fetuses for relative weight, derived from ultrasonography, and for the systolic/diastolic ratio, derived from umbilical artery velocimetry. Both methods distinguish between the two groups. Evaluation of the receiver operating characteristic curves indicate that relative weight is more predictive. When selected points along each curve are evaluated, improved sensitivity, specificity, and predictive values of the positive and negative are evident for relative weight (Tables II and III) and are consistent with the report of Divon et al.[5] Our observations are drawn from a population in which gestational age was known. In a setting when gestational age is not known with certainty, the Doppler assessment may be of value in identifying the SGA fetus.

A systolic/diastolic value of ≥3.0 was found to correlate well with SGA status of the fetus. When maximal sensitivity and specificity were identified, a value of ≥2.85 was determined. This latter value is reasonable when the mean gestational age of studied patients is considered. The upper limit of the systolic/diastolic ratio is 2.9 at 37 weeks and 2.8 at 38 weeks.[12]

The optimal break point for relative weight was 0.784, slightly lower than expected. This reduction may be attributed to the tendency of weight estimation formulas to overestimate SGA fetuses.[13]

The identified differences are obtained in a setting in which SGA fetuses were studied and delivered nearly 2 weeks earlier. The systolic/diastolic ratio decreases with advancing gestational age.[9] One would expect a higher systolic/diastolic ratio in the earlier delivered SGA fetuses. In spite of this bias, relative weight worked better. This observation strengthens the finding that relative weight is superior to the systolic/diastolic ratio.

The study population was enriched with SGA newborns. If a more normal population was selected (prev-

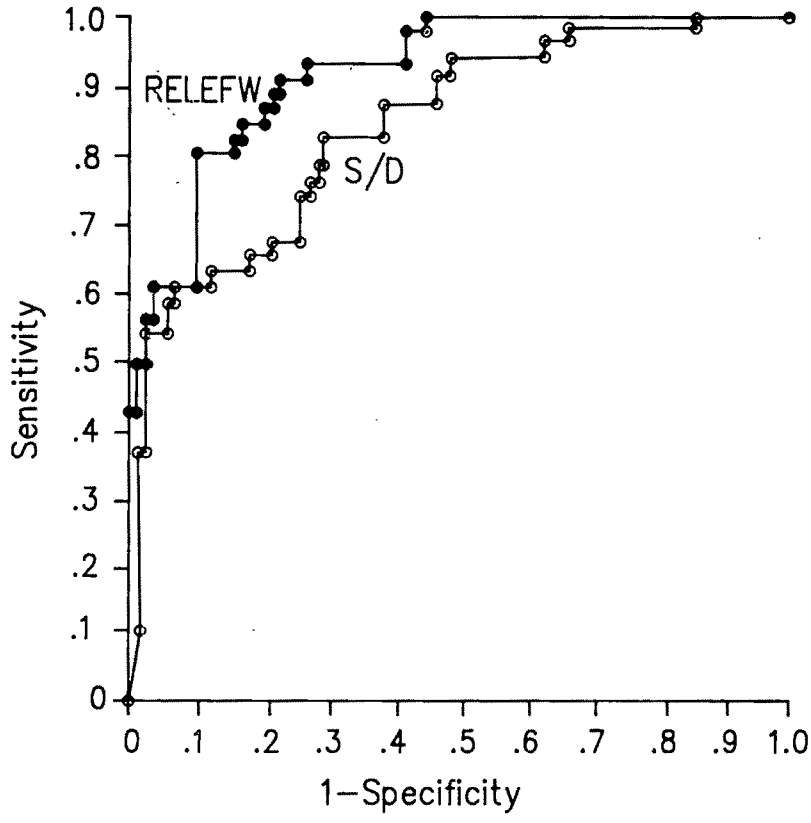


Fig. 1. Receiver-operator characteristic curves of ultrasonographic variables in detection of SGA fetuses. *RELEFW*, Relative estimated fetal weight; *S/D*, systolic/diastolic ratio.

Table II. Contingency tables for ultrasonographic variables versus SGA with a preselected boundary

Variable	*SGA*	*Non-SGA*	*Significance*	*Positive predictive value*	*Negative predictive value*	*Sensitivity*	*Specificity*
Relative estimated fetal weight							
≤0.80	38	13	$p < 0.001$	75%	91%	83%	86%
>0.80	8	77					
Systolic/diastolic ratio							
≥3.0	31	22	$p < 0.001$	59%	82%	67%	76%
<3.0	15	68					

Table III. Contingency tables for ultrasonographic variables versus SGA selected to be closest to upper left-hand corner of receiver-operator characteristic curve

Variable	*SGA*	*Non-SGA*	*Significance*	*Positive predictive value*	*Negative predictive value*	*Sensitivity*	*Specificity*
Relative estimated fetal weight							
≤0.784	37	8	$p < 0.001$	82%	90%	80%	91%
>0.784	9	82					
Systolic/diastolic ratio							
≥2.085	38	25	$p < 0.001$	60%	89%	83%	72%
<2.085	8	65					

alence of SGA 10%) with the boundaries of 0.80 for relative weight and 3.0 for the systolic/diastolic ratio, the predictive value of the positive would decrease to 39.7% and 23.7%, respectively, from application of Bayes' theorem. The predictive value of the negative would increase, 97.9% and 95.4%, respectively; sensitivity and specificity would be unaltered. In a more normal population, both relative weight and the systolic/diastolic ratio would be of value in excluding SGA. In clinical practice, the population specifically studied to detect SGA is often preselected. Therefore assumptions drawn from our data may be appropriate to other settings.

This study addressed SGA newborns as the defined outcome and uses a birth weight–derived definition of SGA. It is reasonable that estimated fetal weight should reflect SGA well. Doppler systolic/diastolic measurements were less useful than standard ultrasonography. The systolic/diastolic ratio may provide insight into the mechanism of diminished growth. Additionally, Doppler measurements may also provide information about fetal health.[14] We concur that their use should be adjunctive.[15]

Clinical management of the SGA fetus is often difficult. We believe that a variety of factors will influence timing of delivery. Serial scans to follow growth of the fetus and some form of antepartal fetal testing for well-being are appropriate. A fetus who fails to grow on serial scans or has an abnormal evaluation of well-being is probably best delivered.

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Discussion

DR. EMANUEL P. GAZIANO, Minneapolis, Minnesota. The article supports the generally recognized concept that umbilical artery Doppler measurements offer no significant advantage over standard ultrasonographic biometry for the diagnosis of the SGA fetus. This discussion will focus on three points: the heterogeneity for any given SGA population, methodologic issues, and clinical implications for the findings presented.

Studies relating to the prenatal diagnosis of the SGA fetus by dissimilar methods need to consider, among other things, cytogenetic, infectious, nutritional, and vascular causes. For example, the fetus shown to be SGA on the basis of cytomegalovirus may not necessarily have increased resistance in the umbilical circulation, a prerequisite for abnormal Doppler velocimetry. Although the preponderance of infants in any given population are SGA on the basis of compromised uteroplacental blood flow, did the authors seek to classify the SGA infants on the basis of cause independent of gross anomalies?[1]

The arbitrary cutoff of 3.0 used as a threshold for abnormality is a concern for two reasons. This value was described for studies conducted with continuous wave rather than pulsed Doppler and it does not consider the critical relationship between gestational age and Doppler values. Depending on sample volume size, Doppler indices obtained with pulsed technique as opposed to continuous wave tend to be higher in the umbilical artery for any given gestational age. In our own population, with pulsed Doppler and a sample volume size of 1.5 mm,[2] all patients with a gestational age ≥29 weeks have mean Doppler systolic/diastolic ratios of ≤2.9, which is in close agreement with those reported by Gerson et al. for pulsed Doppler.[2,3] If one considered ≥2 SDs above the mean as the threshold for abnormality, as is suggested by most investigators, a number of patients would be misclassified by the current

study.[1,3] Accuracy could be improved by the construction of a standard curve from the authors' own population and equipment, with the above criteria for abnormality or by use of published normal values for pulsed Doppler, which are adjusted for gestational age.[3] The authors are to be commended for selecting a formulation for ultrasonographic estimation of fetal weight, which includes femur length and for selecting receiver-operator characteristic curves as the statistical method for displaying data, allowing optimal values to be set for test sensitivity.

Table I needs to be expressed in weeks instead of days for time of study and time of delivery.

The study population is surprisingly advanced in gestational age with a mean of 36.4 ± 3.3 and 38.2 ± 2.0 weeks for SGA and non-SGA infants, respectively. The whole construct of the receiver-operator characteristic curves are based on this population. This point needs to be stressed in the discussion because the selected cutoffs would presumably have little or no validity for the diagnosis of the SGA fetus at ≤33 weeks.

The findings that Doppler velocimetry offers no advantage over ultrasonographic estimation of fetal weight is not surprising because one method measures a physiologic parameter, distal resistance to flow, in a heterogeneous population. In addition, ultrasonographic estimations for weight may be more sensitive for the smaller fetus.[4] Does this leave any role for fetal Doppler or will Doppler be consigned as the estriol test of the 1990s? Emerging clinical studies, supported by the present article, suggest the following: (1) that abnormal velocimetry is highly statistically associated with the birth of an SGA infant, (2) that ultrasonography biometry compared with umbilical artery Doppler is superior for the diagnosis of the SGA fetus, and (3) that abnormal velocimetry correlates best with neonatal morbidity and mortality.

A number of studies, including our own work in multiple gestations, support the latter, suggesting that abnormal Doppler velocimetry, particularly in the presence of lower diastolic velocities, demonstrates a strong association with adverse neonatal outcome.[2]

Abnormal velocimetry of the umbilical artery in the presence of ultrasonography retardation of fetal growth warrants close fetal surveillance. Multiple vessel interrogation may help with the timing of delivery, whereas the presence of oligohydramnios and coexisting maternal hypertension remain important clinical variables. Therefore, in the presence of a growth-retarded fetus, Doppler velocimetry may serve as a useful management tool and will likely complement rather than replace standard ultrasonographic biometry.

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Dr. Dev Maulik, Kansas City, Missouri. This article investigates the comparative efficacy of ultrasonographic estimation of fetal weight and umbilical arterial systolic/diastolic ratio for predicting birth weights <10th percentile. The former technique has been in use for many years, whereas the efficacy of the latter has been extensively investigated in recent years. The use of umbilical arterial Doppler velocimetry for identifying intrauterine growth retardation (IUGR) is based on the rationale that such a complication is associated with and may be the result of a compromised fetoplacental circulation. This was supported by the important observation that a substantial fall in the umbilical blood flow and subsequent fetal growth retardation can be produced by microsphere embolization of the uteroplacental circulation in a ewe model.[1] Early clinical studies indicated a strong association between abnormal umbilical arterial Doppler indexes and suboptimal fetal growth. This led to optimism about the potential of Doppler velocimetry as a powerful diagnostic tool for fetal growth retardation. Subsequent experience, however, has been mixed at best and often not very encouraging. Some of these contradictory findings may be explained by the semantic and conceptual complexities surrounding the terms IUGR and SGA. Indeed, it should be recognized that not all growth-retarded fetuses are SGA, just at not all SGA infants are growth retarded. Furthermore, it is apparent that not all IUGR or SGA fetuses may suffer from in-utero compromise in terms of asphyxia, which correlates better with absent end-diastolic velocity in the umbilical artery than with subnormal fetal abdominal circumference (<5th percentile), as measured by ultrasonography.[2]

In regard to the various ultrasonographic modalities for recognizing an SGA infant, the majority of the studies have demonstrated that conventional fetal ultrasonographic biometry is more sensitive than umbilical arterial Doppler velocimetry (Table I).

Drs. Miller and Gabert's observations are consistent with these findings. However, their investigation has addressed this issue in a more comprehensive manner by using receiver-operator characteristic analysis for the comparison of the two diagnostic modalities. We have expressed our own preference for this analytic approach in this forum in the past.[7,8] Unlike methods based on the distribution-derived threshold value of a test, the receiver-operator characteristic analysis evaluates overall performance of a test in discriminating between the diseased and the healthy population. The area under the receiver-operator characteristic curve offers a general index of accuracy independent of such factors as population attributes, prevalence rate, or the intended use of the test. A comparison of areas under the relative-operator characteristic curves permits a more logical inferential process.

Table I. Comparative efficacy of ultrasonographic biometry and Doppler velocimetry in detecting SGA infants

Series	*Sensitivity (%)*	*Specificity (%)*	*Positive predictive value (%)*	*Negative predictive value (%)*
Divon et al.[3] (1988)	49/87	94/87	81/78	77/92
Berkowitz et al.[4] (1988)	45/79	89/85	58/64	—
Chambers et al.[5] (1989)	47/73	—	77/84	52/67
Gaziano et al.[6] (1990)	79/43	66/98	21/77	96/93
Miller and Gabert	67/83	76/86	59/75	82/91

In conclusion, I congratulate Dr. Miller and Dr. Gabert for their well-conducted study. However, I believe that several points of clarification are in order. First, it is apparent from the high incidence of SGA infants that the study population was at an increased risk. What were the criteria for selecting the patients in this study, and what were the characteristics of the study population regarding pregnancy complications? Second, a critical element in any study of this nature is an accurate estimation of the gestational age. Although it is mentioned in the article about confirmatory early physical examination and ultrasonography, what were the precise criteria? Finally, an earlier study observed that fetal growth compromise may be detected earlier by Doppler velocimetry than by ultrasonographic biometry.[4] Was this phenomenon noted in this investigation?

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DR. CHIN-CHU LIN, Chicago, Illinois. I agree with Dr. Gaziano's opinion that the Doppler study probably looked for the functional rather than anatomic diagnosis of IUGR fetuses by conventional ultrasonographic studies. So it's a different parameter.

From previous literature, it has been suggested that Doppler study for predicting the outcome of the IUGR fetus is not better than various antenatal fetal testings. That includes nonstress testing, biophysical profile, and other testing parameters. My questions are, have the authors compared this Doppler study with those fetal testing parameters? Is this Doppler study better correlated with the diagnosis of IUGR than other antenatal fetal testing methods?

DR. JOE BRUNER, Nashville, Tennessee. From a common sense point of view, simple identification of the SGA fetus is of little interest. Most textbooks point out that 40% of small infants are simply genetically determined to be small. Just like there are big people and little people, there are small infants who are otherwise normal. They're of little interest.

Furthermore, 10% of small infants have severe chromosomal or congenital disorders, and their management is challenging only to a select few. Another 10% are small because of early perinatal infections, and there's little that we can do after the fact to improve outcome in this group.

However, 40% of small babies are small as a result of uteroplacental insufficiency. This is the group we should be most interested in. This group could be identified by a combination of both standard ultrasonographic biometry and Doppler ultrasonography. We should be interested in this group because, in spite of electronic fetal monitoring and biophysical profiles, many of these babies are still born with severe neonatal complications.

So identification of the subgroup of small babies *with* uteroplacental insufficiency should be the starting point for studies trying to decide how best to manage these pregnancies.

DR. DANIEL RIGHTMIRE, Springfield, Illinois. I would like to ask the question that the other two persons from the floor just referred to but didn't ask, and that is, were there different morbidities actually identified among the groups declared abnormal by the two techniques, differences besides smallness? For example, were there differences in the duration of newborn intensive care unit stay or the need for respiratory care or operative deliveries for fetal distress?

DR. MILLER (Closing). Our study population was defined to avoid certain types of complications including cytogenetic or chromosomal abnormalities.

The cutoff point of 3.0 for Doppler studies is prob-

lematic. Our patient population was nearly at term. We chose 3.0 as an arbitrary cutoff; and whether it is right or wrong, it is widely replicated in the literature.

I do agree that Doppler values should be adjusted to gestational age. We chose not to do that since in our community the values are reported as an absolute value compared in that manner.

I concur on the comments with the systolic/diastolic boundaries and their role as gestational age.

Dr. Maulik pointed out the difference between IUGR and SGA, and I could not concur more wholeheartedly. We defined the population as being SGA because that was a relatively easy patient population to define. The problem with defining an IUGR is that I'm not aware of a single widely accepted definition that I could then use to study prospectively. I beg for cooperation from those in the audience to provide such a definition, and we will be happy to use it.

The characteristics of our study population were not presented. We run a consulting service and deal almost entirely with patients who are referred for evaluation and then returned to the practicing physician. The population was indeed rich in SGA newborns, which is not surprising because we were looking specifically to study them. To be sure, chronic hypertension, preeclampsia, poor weight gain, smoking, and other underlying medical complications such as renal disease and lupus erythematosus were among these. These were not recorded.

By early physical examination we meant first-trimester examinations. The patients were all referred by practicing physicians, and patients had been enrolled in prenatal care early. Ultrasonography was generally obtained first, in the first trimester, and if not then, by 16 weeks so that we could define the gestational age. We recognize that there will be minor variations within this. It's unavoidable because we don't have documented ovulation for these patients.

I cannot comment on early growth changes and the ability to determine SGA babies at 24, 28, or 32 weeks because that was not the intent of this study. We do have such data and ultimately will be able to pull them out.

There were some concerns about the functional versus anatomic changes that are being reported. We did not compare these because that was not in our study design, biophysical profile to Doppler. This would be a very interesting study.

I concur that it would be a mistake to walk away from this study believing that Doppler is not of use. Clearly it has a role. I'm waiting for its full role to be defined.

In our study population, however, there was a difference between the two areas under the curve, even though both were excellent, 84% and 92% being reported. The difference in areas under the curve was statistically significant, and that is an important finding from our study. We did not find "no difference." We found specifically that if you want to identify a small baby ultrasonography does so better than Doppler.

Dr. Bruner asked about better identification and the role of Doppler in the IUGR baby. My comments from before will stand.

One would be concerned about the heterogeneous population that we reported. Indeed, my own thoughts are there are several categories of small babies. I see many small babies who weigh 5 pounds to 5 pounds 12 ounces at term and who have grown at a very normal rate over the third trimester. They generally have good amniotic fluid and biophysical assessment, either nonstress test or biophysical profile. These patients are generally delivering smaller but otherwise entirely normal babies.

These are quite different from babies who have a dysfunctional growth process with decreased amniotic fluid, abnormal biophysical profiles, or other abnormal antepartum results.

I would ask that others join us in our efforts to try to differentiate among these entities and to develop a protocol to better focus on both diminished and abnormally diminished growth.

Prediction of respiratory distress syndrome by a new colorimetric assay

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Albany, New York, and Savannah, Georgia

OBJECTIVE: The purpose of this study was to evaluate the clinical performance of a new, simple, and rapid colorimetric assay for predicting respiratory distress syndrome.

STUDY DESIGN: Ninety-four specimens obtained within 3 days of delivery were assayed with the new test. For 78 of these specimens, the lecithin/sphingomyelin ratio was also determined. Significant differences were calculated with χ^2 analysis. Cutoff points were selected by maximizing the corresponding sums of sensitivity and specificity and from receiver-operator-characteristic curves.

RESULTS: The specificity of the new colorimetric test was better than 89%, and it yielded more than 86% correct diagnoses. Furthermore, the test had a significantly ($p < 0.05$) lower percentage of false-immature results than that obtained with the lecithin/sphingomyelin ratio. Moreover, the new test performed relatively well in analyses of patients between 27 weeks and 36 weeks' gestation.

CONCLUSION: We concluded that the new assay is reliable and that it can serve as a useful alternative to current methods for predicting respiratory distress syndrome (Am J Obstet Gynecol 1992;166:1827-34.)

Key words: Fetal lung maturity, respiratory distress syndrome, colorimetric test for fetal lung maturity

Respiratory distress syndrome (RDS) is still the leading cause of morbidity and mortality among premature newborns.[1] Most methods for predicting RDS are based on the well-known relationship between specific phospholipids in amniotic fluid and progress in fetal lung maturity.[2] The most commonly used clinical test, the lecithin/sphingomyelin (L/S) ratio,[2,3] is labor intensive, time consuming, and generally performed by a clinical technician with special training. Two anionic phospholipids, phosphatidylinositol and phosphatidylglycerol, were suggested by Kulovich and Gluck[4] as additional indexes for fetal lung maturity. An advantage of using these phospholipids is that their low levels in blood should improve the analysis of blood-contaminated amniotic fluid specimens. Phosphatidylglycerol is determined generally by a complex, two-dimensional, thin-layer chromatography procedure[2] or by an immunoagglutination test.[5] This phospholipid is usually detected late in gestation; its estimation is used therefore as a screening test.[2,6] The determination of a phospholipid profile in amniotic fluid may increase the clinical performance over that of the L/S ratio,[2,4] but the procedure is much more labor intensive.

We recently reported on a simple and rapid colorimetric assay for simultaneous determination of lecithin, phosphatidylinositol, and phosphatidylglycerol in amniotic fluid.[7] The assay is based on the capabilities of these phospholipids to solubilize a solid dye complex adsorbed on glass beads. We simplified the original procedure and made it more convenient for clinical use. In this communication we describe the improved assay and report on a clinical evaluation of the new test in assessing fetal lung status.

Material and methods

Phospholipids for calibration curves were purchased from Sigma Chemical Company (St. Louis). Amniotic fluids were obtained by amniocentesis or aspiration of fluid in the course of cesarean delivery for accepted obstetric indications from patients of the obstetric services of the Albany Medical Center Hospital. Samples with blood contamination and those that were very cloudy after centrifugation were excluded. The results obtained with the new test were not available to the physician determining fetal respiratory outcomes. Neonatal respiratory distress was determined on the basis of neonatal requirements for supplemental oxygen for at least 24 hours and characteristic chest radiographs (presence of a diffuse reticular-granular pattern and air bronchograms). Transient tachypnea of the new-

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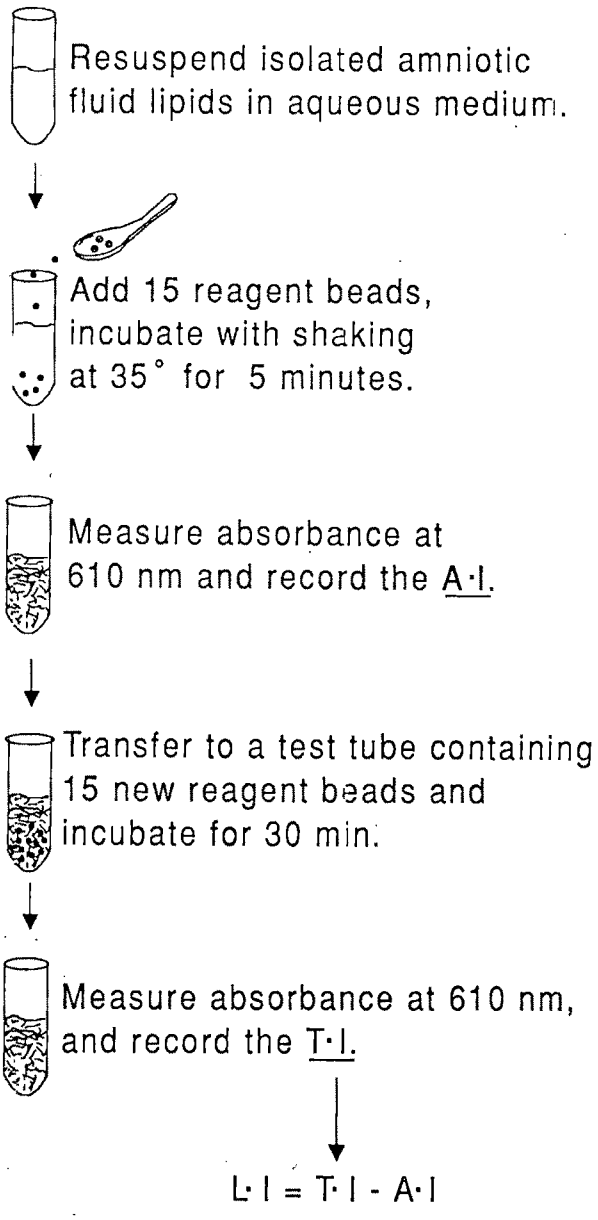


Fig. 1. Schematic for analysis of amniotic fluid specimen with modified test (differential incubation procedure).

born was determined by requirement for <12 hours of ventilatory assistance and the absence of radiographic findings.

One hundred seventeen patients were tested with the new test. The clinical performance of the assay was assessed from diagnoses of 94 amniotic fluid specimens (RDS prevalence of 16%) that were collected within 3 days of delivery. Gestational ages, which were evaluated from menstrual history, ultrasonography, and Dubowitz scores, ranged between 27 and 40 weeks. Nine of the newborns had transient tachypnea of the newborn; seven patients had premature rupture of membranes and 25 were diabetics. Of the 94 patients, 78 (RDS prevalence of 18%) were analyzed by thin-layer chromatography and the L/S ratio was determined.

Fetal lung maturity assessment. Phospholipids were extracted from 1.5 ml amniotic fluid that was centrifuged at 200*g* for 5 minutes by two volumes of chloroform and one volume of methanol. The organic phase was placed in a 16 × 125 mm test tube and the solvent was removed by evaporation under nitrogen. The phospholipid residue of the organic phase was resuspended in 2.0 ml water by a 2-minute vortexing.

Reagent-glass beads were prepared by using a dye reagent precipitated from a mixture of 8×10^{-5} mol/L cetyltrimethylammonium bromide and 5.5×10^{-5} mol/L coomassie brilliant blue. The blue precipitate was dissolved in 5 ml acetone, and the solution was added to 50 gm of 3 mm glass beads (Kimax, Kimble Science Products, Vineland, N.J.). The reagent was adsorbed onto the beads by evaporating the solvent to dryness.[7]

A scheme for amniotic fluid analysis for fetal lung maturity is shown in Fig. 1. Fifteen reagent beads were added to a 2.0 ml sample and incubated for 5 minutes at 35° C with 250 rpm shaking. The absorbance of the blue solution at 610 nm was measured and represented as A·I. The solution was then transferred to another test tube containing 15 new reagent beads; incubation continued for another 30 minutes. The absorbance at 610 nm at the end of this interval reflected T·I. L·I was obtained by subtracting the value of A·I from that of T·I.

Thin-layer chromatography for the L/S ratio determinations was performed as before.[7] L/S ratio >2.0 was taken to indicate maturity. In general, thin-layer chromatography of the lipid extract of the amniotic fluid sample was performed first. The fluid was then stored at −20° C, up to several weeks, before analysis by the colorimetric assay. Results are a mean of duplicated determinations.

A Beckman CDS-200 densitometer was used for the L/S ratio determination and a Perkin Elmer Lambda 4B (Perkin Elmer Corp., Norwalk, Conn.) spectrophotometer for absorbance measurements. Statistical analysis and clinical performance evaluations were performed with the statistical capabilities of RS/1 and an IBM-compatible microcomputer. Variation is indicated by standard deviation. Significant differences were determined by χ^2 analysis, and for small sample size the χ^2 statistic with correction[8] was used.

Results

Fetal lung maturity was assessed with an improved procedure based on the specific solubility of a novel dye reagent in an amniotic fluid phospholipid sample with two incubation intervals. The modified assay circumvented the use of organic solvent required previously[7] for the simultaneous estimation of lecithin and anionic phospholipids, such as phosphatidylinositol and phosphatidylglycerol, in amniotic fluid. Reagent-glass beads were incubated in a water suspension

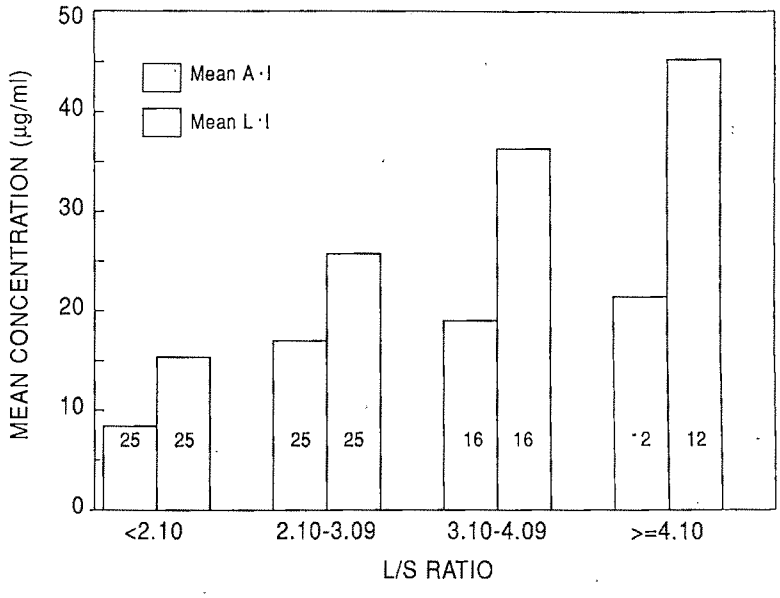


Fig. 2. Correlation between results of new assay (A·I and L·I) with different ranges of L/S ratio. Number of patients tested at each L/S ratio range indicated inside *bars*.

of isolated amniotic fluid lipids for 5 minutes and 30 minutes (Fig. 1). The anionic phospholipids were estimated after the first incubation interval, whereas the lecithin content was measured after the second interval (Fig. 1).

The absorbance values ranged between 0.14 and 0.75 for 95% of the specimens tested in this study. The mean coefficient of variation percentages for the 610 nm absorbance measurements at each incubation interval within a run at different levels of amniotic fluid maturity were 6.9% ± 1.7% and 9.6% ± 2.0% and between runs 6.6% ± 1.5% and 8.6% ± 1.8%, respectively. Proteins, such as unlipidated bovine serum albumin (50 μg/ml), cholesterol (50 μg/ml), lysophosphatidylcholine (20 μg/ml), and sphingomyelin (20 μg/ml) produced very little absorbance (<0.05) under the conditions of the assay.

Absorbance values of the new colorimetric test were converted to phospholipid concentration with appropriate calibration plots determined for each batch of reagent-glass beads preparation. Three clinical indexes—A·I (anionic phospholipid index), L·I (lecithin index), and T·I (total phospholipid index)—were determined simultaneously. The relation between A·I and L·I with different L/S ratios was illustrated in Fig. 2. A trend of increasing index values with the L/S ratio was evident. Nine patients with L/S ratios (1.5 ± 0.4) indicating immaturity had a corresponding mature A·I (14.4 ± 5.2 μg/ml). Just one of these patients had RDS. Eight patients had immature L/S ratios (1.5 ± 0.4) and a corresponding mature L·I (23.5 ± 5.7 μg/ml). Two of these patients had RDS.

Diagnostic cutoff points for A·I and L·I were obtained with 94 specimens that were collected within 3 days of delivery. Cutoff points were selected by minimizing the number of false results in predicting respiratory outcome, by maximizing the corresponding sum of sensitivity and specificity, and by a receiver-operator characteristic curves analysis[8, 9] (Fig. 3). To define a positive test as predictive of RDS,[10] the selected optimal

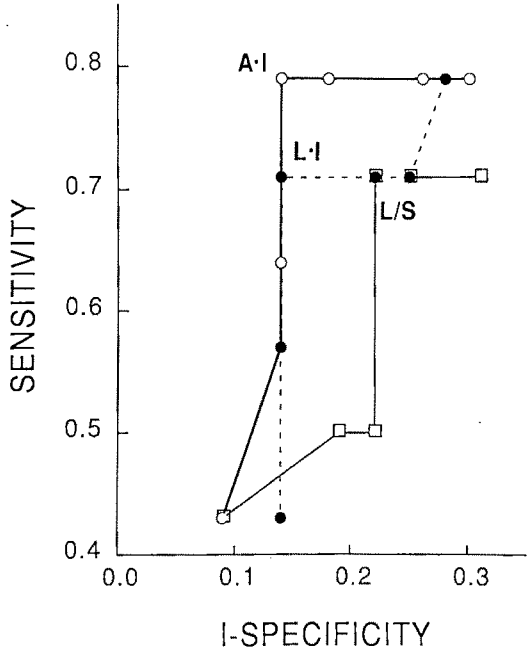


Fig. 3. Receiver-operator-characteristic curves for A·I and L·I of new assay and for L/S ratio. Sensitivity and 1-specificity were calculated at various cutoff points, and respective optimal cutoff points are shown. (Number of samples analyzed by both methods were same [n = 78].)

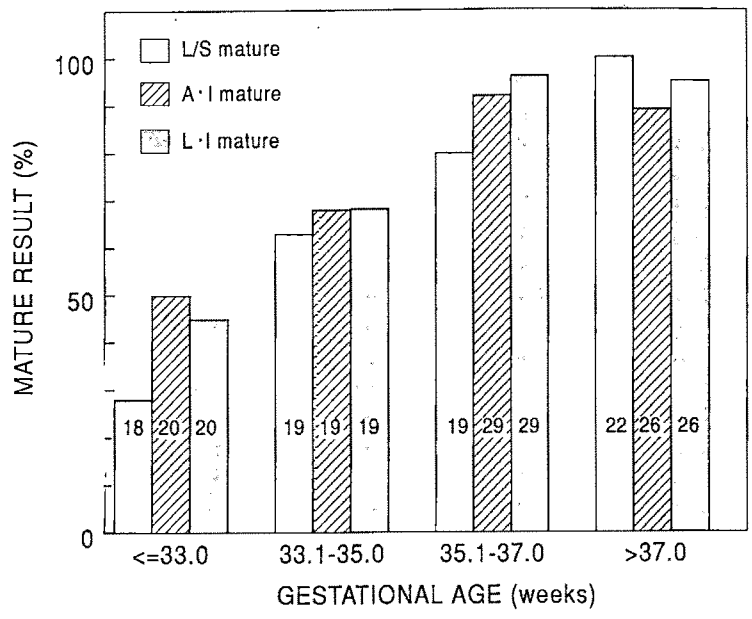


Fig. 4. Correlations of new test results and L/S ratio with gestational age. Number of patients assayed with each test at each gestational age range are indicated inside *bars*.

Table I. Clinical performances of new assay (n = 94) and L/S ratio (n = 78) in predicting RDS

*Clinical parameter**	*Diagnostic index*			
	A·I	*L·I*	*AL·I†*	*L/S*
Specificity	89 (86)	89 (86)	93 (92)	78
Sensitivity	80 (79)	73 (71)	73 (71)	71
Negative predictive value	96 (95)	94 (93)	96 (95)	93
Positive predictive value	57 (55)	55 (52)	71 (69)	42
Efficiency	87 (85)	86 (83)	90 (90)	77
False-positive results (%)	11 (14)	11 (14)	7 (8)	22

Diagnostic indexes A·I, L·I, and AL·I are defined in the text. Values in parentheses were obtained with the L/S ratio study population (n = 78).

**p*, Positive for RDS; *N*, negative for RDS; *FP*, immature result and no RDS; *TP*, immature result and RDS; *FN*, mature result and RDS; *TN*, mature result and no RDS; specificity = $\frac{TN}{TN + FP} \times 100$; sensitivity = $\frac{TP}{TP + FN} \times 100$; negative predictive value = $\frac{TN}{TN + FN} \times 100$; positive predictive value = $\frac{TP}{TP + FP} \times 100$; efficiency = $\frac{TP + TN}{All} \times 100$; percentage of false-positive results = $\frac{FP}{FP + TN} \times 100$.

†A positive test with AL·I was indicated only if both A·I (≤7.5 μg/ml) and L·I (≤19.0 μg/ml) were below their cutoff points.

cutoff values were 7.5 and 17.0 μg/ml phospholipid, respectively. Fifty-four specimens were treated by the differential-incubation assay, and 40 specimens were analyzed by the earlier procedure.[7]

The percentage of mature results (samples with values higher than cutoff points) assessed by both the new indexes and by the L/S ratio increased with gestational age (Fig. 4). The percentages of healthy newborns, as determined by respiratory outcomes, at each gestational age group were 45% before 33 weeks, 84% between 33 and 35 weeks, 96% between 35 and 37 weeks, and 100% after 37 weeks' gestation. Before 33 weeks' gestation, the percentage of mature results with the new test (Fig. 4) was in excellent agreement with the outcome data. In contrast, the percentage of mature results according to the L/S ratio was much lower than the actual percentage of healthy outcomes. This is consistent with the large number of false-positive results that were obtained with this ratio before and at 33 weeks' gestation.

The diagnostic performance of the new test was evaluated by determining the specificity, sensitivity, predictive values, efficiency, and percentage of false-positive results (Table I). The diagnostic efficiency of all indices

Table II. Effect of number of days between specimen collection and delivery on clinical performances of new assay (A·I) and L/S ratio (parameters defined in Table I)

	No. of days within delivery					
	2		*3*		*4*	
Clinical parameter	*A·I (n = 81)*	*L/S (n = 68)*	*A·I (n = 94)*	*L/S (n = 78)*	*A·I (n = 117)*	*L/S (n = 98)*
Specificity	89	78	89	78	88	77
Sensitivity	80	71	80	71	82	73
Negative predictive value	94	91	96	93	96	95
Positive predictive value	61	45	57	42	51	34
Efficiency	88	76	87	77	89	78

Table III. Clinical performances of new assay and L/S ratio in predicting RDS before and at 36 weeks' gestation and in a region of transitional L/S ratios

	Gestational age ≤36 wk		*2.5 ≥ L/S ≥ 1.6*	
	AL·I (n = 39)	*L/S (n = 37)*	*AL·I (n = 22)*	*L/S (n = 22)*
Efficiency (%)	77	59	82	50
False-positive results (%)	16	46	13	53

The performance of AL·I (parameters defined in Table I) is compared with that of the L/S ratio.

was above 86%, and the specificity was above 89%. The specificity and the predictive value of an immature (positive) result improved when a combined index of A·I and L·I, designated as AL·I (RDS was predicted only if both A·I [≤7.5 μg/ml] and L·I [≤19.0 μg/ml] were below their cutoff points), was used (Table I).

The clinical performance of the new test was compared with that of the L/S ratio by evaluating the results obtained from analyses of amniotic fluid specimens with both tests ($n = 78$). The specificity and the predictive value of a positive result were higher for the new test than with the L/S ratio (Table I). The number of false-positive results was significantly ($p < 0.02$) lower for the AL·I (8%) than for the L/S ratio (22%) (Table I).

The receiver-operator characteristic curves of the new indices and the L/S ratio crossed below 0.5 sensitivity, and the portion of the curve of greatest clinical interest[8] is shown in Fig. 3. The new test curves were closer to the upper left corner of the graph than the L/S ratio curve, suggesting that the new test is superior. The 1-specificity (false-positive rate) of the new test was compared statistically with that of the L/S ratio at a fixed sensitivity with paired samples.[8] At 0.79 sensitivity the corresponding false-positive rates were 0.14 with the A·I (≤7.5 μg/ml) and 0.31 with the L/S ratio (<2.5), indicating that the new test performed better ($p < 0.03$) than the L/S ratio.

The clinical performance of the new assay changed very little with samples collected within 2 or 4 days of delivery (Table II). In all but one of the false-negative results, RDS was classified as mild, and the pregnancies had at least one other complication including twin, preterm labor, amnionitis, premature rupture of membranes, or preeclampsia. These patients also had a mature result by optical density measurements ($OD_{650} > 0.15$) with a mean OD_{650} of 0.38 ± 0.16.

The test performed well under demanding conditions. The clinical performances of the new test and the L/S ratio, in patients up to and including 36 weeks' gestation, are given in Table III. The number of false-positive results was almost threefold lower with the AL·I than that obtained with the L/S ratio (Table III). The AL·I was significantly better ($p < 0.05$) than the L/S ratio in indicating maturity. The specificity and sensitivity of the test were 84% and 71% as compared with 54% and 69% for the L/S ratio. In addition, for patients with transitional L/S ratios between 1.6 and 2.5, the percentages of false-positive results were 13% and 53% with the AL·I and the L/S ratio, respectively (Table III).

Comment

The new colorimetric test for antenatal assessment of fetal lung maturity is based on the specific solubility of a single-solid dye reagent, adsorbed on glass beads, in an aqueous solution of amniotic fluid phospholipids. This reagent can be stored at room temperature for months and eliminates the problems associated with labile biologic reagents. The new test can be performed

by someone without extensive technical expertise, takes less than an hour to complete, and is quantitative and cost-effective.

The ability to express results quantitatively may contribute to improving the prevailing poor interlaboratory reproducibility in predicting RDS. The sum of L·I and A·I of a mature result was higher than 25 μg/ml phospholipid, in agreement with previous reports.[11] The cutoff points ratio (L·I/A·I) was 2.3, which corresponds to about 70% lecithin and 30% anionic phospholipid.

Fetal lung maturity assessment is complex and involves many complicating factors. Many of the widely used methods for predicting RDS are based on the measurement of a single component or property of lung surfactant. The new test is based on both the surface properties and the phospholipid composition of lung surfactant. At least two different diagnostic indexes corresponding to different classes of phospholipids were determined. The performance of the test is expected to be enhanced by its provision for quantitative and simultaneous determination of more than one amniotic fluid phospholipid index, as shown by a relatively high predictive value of an immature result (Table I).

The clinical usefulness of the new test was expressed by a high specificity, a high percentage of correct diagnoses, and a significantly lower percentage of false-immature results than that obtained with the L/S ratio. This suggests that this new promising test may be a good alternative to current methods for predicting RDS.

We thank Donald S. Berns for his assistance, Paul Sherman for performing the thin-layer chromatography measurements, Bernard R. Lipinskas for his help in installing our computer, and Andrew A. Reilly for his help with the statistical analysis.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

Dr. Charles M. Stedman, New Orleans, Louisiana. I distinctly recall the clinical laboratory class that day at medical school in 1972. The instructors had chosen the L/S ratio as the project for the afternoon, and we were the first class to participate. The test had been introduced in 1971 by Gluck et al.,[1] who reported a technique to screen for fetal pulmonary maturity. We should all pause to consider that this article is one of those rare medical works that has continued as the gold standard 20 years after initial presentation.

The impact of the L/S ratio in the 1970s was as a reliable screening test. Obstetricians finally had a tool to prevent instances of iatrogenic prematurity at repeat cesarean section. The pediatric literature of the day described unintended prematurity accounting for 10% to 15% of admissions to intensive care nurseries. During a time when urine pregnancy tests were relatively insensitive, ultrasonography was used sparingly, and scheduled repeat cesarean section was the norm, the L/S ratio served as a safeguard. Its clinical utility was to predict the absence of RDS.

In the 1990s our needs are somewhat different. Use of the L/S ratio as a screening test is less frequent. Urine pregnancy tests are positive when a menstrual period is 1 day late and first-trimester sonograms establish the gestational age within narrow confidence limits. In spite of some physicians thinking vaginal birth after previous cesarean section means "very bad alternative to cesarean," many patients are allowed to labor.

Currently it is my observation that we use fetal lung maturity testing more as a semiquantitative test than as a screening test. In cases of fetal distress we attempt to weigh the likelihood of RDS and the associated complications against the risk of continued in utero existence. When we have patients experiencing preterm labor, our knowledge of the L/S ratio can affect the level of intensity of tocolytic treatment. Harvey et al.[2] have reviewed 385 patients delivered within 72 hours of sampling. For values between 1.5 and 2.0, 40% developed RDS; for values <1.5, 73% developed RDS. In most laboratories, an L/S ratio <1.0 assures RDS. Unfortunately, these numbers are variable from institution to institution because of the interlaboratory variability of the ratios.

Almog and associates have described a technique with the apparent advantages of being less labor inten-

sive; less time-consuming, more reproducible from laboratory to laboratory, and more convenient to the obstetrician because of the smaller sample size required. The critical portion of this article concerns the fewer false positives (i.e. false immature results) in the <33 weeks group. Fifty percent of samples were mature by the anionic index, yet only 28% were mature by the L/S ratio. Dr. Almog provides us with the hope of identifying more of the fetuses who will not develop RDS. We could spare more women from what I call brutal tocolysis. I am referring to patient who has developed premature labor recurrently and may require multiple courses of intravenous tocolytics with all the associated side effects.

To critically analyze the <34 weeks group, I think obstetricians would like to know more clinical details. What were the corresponding L/S ratios for the patients mature by the anionic index but immature by the L/S? Were most 1.8 to 1.9 or were they much lower? Was gestation age assigned by early ultrasonography or by Dubowitz scores? What percentage of patients had received glucocorticoids to enhance pulmonary maturity?

Certainly amniotic fluid analysis is not mandatory before all preterm deliveries. The pregnant woman with severe hypertension is delivered for maternal reasons. The delivery of a fetus with severe growth retardation and reversed Doppler flow in the cord is not going to hinge on the L/S ratio. It's the patients at 32 to 34 weeks requiring repetitive or intense tocolysis that are difficult for all of us.

In summary, this manuscript was well written and targeted a clinical area where improvement is needed. My only major criticism is that 40 of 96 samples were analyzed by the previously reported phase-partition procedure and lumped with 54 samples analyzed by the newer method. Such a change in methodol makes it difficult to apply statistical analysis. Nevertheless, the false-immature values in the <34 weeks group were much less frequent using the anionic index. I find myself feeling like most fans of the 5-0 New Orleans Saints—the initial percentages are exciting, but we need more numbers before allowing unbridled enthusiasm.

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DR. ROBERT C. KAUFMANN, Springfield, Illinois. The authors of this paper have made claims that have a high probability of not being true. This has occurred because of a study design that is at risk for a type 1 error and because of a lack of proper presentation and analysis of data. The authors should not feel lonely, because this seems to be a common problem with many papers.

The receiver-operator curves for this paper are misleading in that the scales of the x and y axes are not equal. What appears to be an obvious difference in the receiver-operator characteristic curves vanishes when the scales are equalized. In fact, statistical analysis of these curves reveal that they are essentially identical with the area underneath the A·I, L·I, and L/S curves, 0.15, 0.12, and 0.12, respectively, and the standard error of each curve is 0.04. Therefore the receiver-operator curves do not illustrate the superiority of the new indices.

In the design of this study the results of the L/S ratio were available to the obstetrician for use in the management of the patient, whereas the results of the new indices were not available. This leads to verification bias, which is inherent in most studies of "new" methods to predict RDS. (1) Because most babies that have low L/S ratios will not be delivered, (2) because most babies that have high L/S ratios will be delivered, and (3) because only those cases with delivery within 3 days of specimen collection are analyzed, there is a bias for delivering those infants who have negative test results over those who have positive test results. Such bias causes the false-positive and false-negative rates of the L/S to be overestimated and those of new indices to be underestimated.[1] By using the undelivered cases one can mathematically correct for verification bias. In the interest of time and to not totally bore the majority of this audience, the details of these corrections will not be pursued. Suffice to say that such a lack of correction for verification-bias causes the conclusions of this study to be suspect. Those desiring to pursue this matter are encouraged to read an article by Hunink et al.[1] entitled, "Testing for fetal lung maturity."

The authors have further compounded the error of verification bias by an error in study design. There is an inequality in the number of cases and the rate of RDS in the cases that were analyzed by the two methods. There were a total of 94 cases analyzed by the new method, although only 78 of these were analyzed by the traditional L/S method. In these 78 cases the rate of RDS was 18% (14 cases), whereas in the remaining 16 cases analyzed only by the new indices the rate of RDS was only 6% (one case). Although these rates are not significantly different, the addition of these 16 samples to L/S analysis could significantly change the results and conclusions of this study. If, in these 16 patients, the L/S ratio correctly predicted the one case of RDS (the probability of correct prediction is 71%) and correctly predicted all of the non-RDS cases (a probability of 78%), the L/S ratio would have statistically similar results as all of the new indices. Such a finding would cause the author to state that the new indices are not superior in predicting either RDS or the lack of it.

Although I have been somewhat critical of this paper, I do not want Dr. Almog and his colleagues to feel lonely. We are having to deal with the same problems of study design and verification-bias in trying to develop a "new" test for fetal lung maturity in our institution. Although little is made of it in the paper, the author raises a point (one we have also begun raising) that sometimes we want a test that minimizes the risk

of RDS and other times we want a test that maximizes the lack of risk for RDS. The combination of the new indices that the author has proposed may be helpful in this regard. I would like to encourage the authors to continue to pursue this research and to try to correct the errors that I have elucidated. Even if the new indices are only equal to the traditional L/S ratio in predicting RDS but they can both minimize the risk of RDS and maximize the lack of risk for RDS, the simplicity and reproducibility of the new method could prove to be a valuable tool for clinical use.

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Dr. Daniel Rightmire, Springfield, Illinois. I would like to know if the authors have any information about the performance of their test in the presence of meconium, bilirubin, or oxyhemoglobin pigments.

Dr. Almog (Closing). The purpose of this manuscript was to catalyze and encourage the use of the test in clinical laboratory settings. I agree with Dr. Stedman that the test should be studied further before it is used in the management of patients. I also agree that the most critical gestational age group is 32 and 33 weeks. Later on, RDS is not as important and it is not a critical issue in patient management.

I thank Dr. Kaufmann for his very constructive criticism. The scales of the receiver-operator characteristic figure are somewhat misleading, but they do not affect the relative location of each curve. I did not calculate the areas under the curves but I am going to look into it. However, the specificity and the sensitivity show that our test is at least as good if no better than the L/S ratio.

Concerning the question of different population sizes, the performance of the new test, using the L/S ratio population, was still significantly better than the ratio in indicating maturity. This was also indicated in the slide describing the performance of both tests before 36 weeks' gestation.

The bias that may result from the fact that the L/S ratios were known to the physicians and our test results were not known is a problem that anyone developing a new test has to face. However, both tests were performed on the same high-risk heterogeneous population. Each clinical complication may affect the verification and occurrence of RDS differently. The L/S ratio was not consulted in many cases and was one of many factors that were taken into consideration in the decision making of patient management. In this situation verification bias analysis is complex, and the outcome of such analysis may not be clear.

A question was asked about the performance of the test in the presence of meconium, bilirubin, or bloodstain. Very little was done in this area but we did analyze several contaminated samples and the test performed quite well.

It should be noted that the test measures the amount of anionic phospholipids. Anionic phospholipids are not present, except in very small amounts, in meconium and blood, whereas lecithin and sphingomyelin, which are used in the L/S ratio, are present in these fluids. Therefore our test should perform much better than the L/S ratio, but we have not studied as many samples as we wish to study.

Urinary incontinence: Correlation of history and brief office evaluation with multichannel urodynamic testing

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OBJECTIVE: Patients' histories of urinary incontinence and the results of several standard clinical tests were correlated with final diagnoses obtained by multichannel urodynamic testing. We used a combination of clinical test results to predict the final diagnoses.

STUDY DESIGN: Ninety consecutive women with a primary complaint of urinary incontinence completed a standardized questionnaire and underwent a structured clinical examination consisting of several standard clinical tests. Each patient later underwent multichannel urodynamic testing to obtain a final diagnosis.

RESULTS: Although the symptoms of stress incontinence were significantly associated with genuine stress incontinence and mixed incontinence, overlap in patients with detrusor instability did not allow the histories to be useful diagnostically. The only clinical tests showing significant association with the final diagnoses of incontinence were the cough stress test and single-channel medium-fill cystometry. Reliable prediction of the urodynamic diagnosis of incontinence could not be achieved with either of these two tests or with a combination of variables obtained by discriminant analysis.

CONCLUSIONS: Women with complaints of urinary incontinence, especially those for whom surgery is contemplated, should undergo complete urodynamic evaluation when it is available. (Am J Obstet Gynecol 1992;166:1835-44.)

Key words: Urinary incontinence, urodynamic testing

The treatment of a female patient with a primary complaint of urinary incontinence is often based on history alone. Because history correlates poorly with the objective cause of urinary incontinence, therapy directed by a patient's complaints can be incorrect.[1-6] However, when the clinician performs an objective evaluation of incontinence, he or she may be unfamiliar with the methods of office testing or may refer the patient for highly technical and expensive urodynamic testing.

We present our investigation of urinary incontinence, in which we studied the correlation of the patient's history and several selected widely available clinical tests with the final diagnosis of urinary incontinence determined by multichannel urodynamic testing. The objective of this investigation was to correlate the patient's history of incontinence with the final diagnosis on the basis of urodynamic testing and to compare this urodynamic diagnosis with results of several clinical tests that can be performed in any practitioner's office. In addition, we attempted to make a predicted diagnosis of urinary incontinence that was based on selected clinical tests, and we compared this diagnosis with that obtained by multichannel urodynamic testing. Our aim was to determine what should be included in a minimum office evaluation and to identify those patients who require multichannel urodynamic testing.

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Materials and methods

This prospective study was begun in September 1987 at the University of California, Irvine and Memorial Medical Center, Long Beach, and was continued after July 1988 until June 1991 at the University of Tennessee, Memphis.

Ninety consecutive female patients were evaluated for a primary complaint of urinary incontinence. Their ages ranged from 21 to 76 years (mean 52.8 ± 12.0 years) and the mean parity was 3.1 ± 1.8 (range 0-10). The racial distribution showed 72 white patients (80.0%), 16 black patients (17.8%), and 2 Hispanic patients (2.6%). At the first visit all patients gave a detailed history and underwent a standardized group of clinical tests that resulted in a preliminary diagnosis of incontinence.

In addition to demographic data, the history included a 24-hour voiding diary and a group of ques-

Table I. Final diagnoses for 90 patients determined by multichannel urodynamic testing

Urodynamic diagnosis	*Frequency*	
	No.	*%*
No incontinence	8	8.9
Genuine stress incontinence	38	42.2
Detrusor instability	17	18.9
Mixed incontinence	24	26.7
Urethral instability	2	2.2
Overflow incontinence	1	1.1
TOTAL	90	100

tions that were selected from our standard questionnaire[7] to differentiate genuine stress incontinence from detrusor instability or other disorders of overactive detrusor function. Questions were read directly from the data recording sheet to avoid questioning bias. The questions were as follows:

1. Do you lose urine during coughing, sneezing, or vomiting?
2. Do you lose urine with walking, running, straining down, or changing positions?
3. When you are passing urine, can you usually stop the flow?
4. Do you lose control and pass a large amount of urine when you cough, sneeze, or strain?
5. Do you always have an uncomfortably strong need to pass urine before you empty your bladder?
6. Do you lose urine before reaching the toilet?
7. How many times do you void during the night after going to bed?

Questions 1 and 2 were thought to predict genuine stress incontinence and questions 3 to 7 were considered to predict detrusor overactivity.

The clinical examination and tests were based on a standard evaluation used in our urogynecology clinic but were structured so that clinicians could perform these tests in their offices with minimal equipment and previous instruction. The clinical evaluation included a catheterized urine sample used to determine residual volume and to perform culture, neurologic and pelvic examinations, a Q-Tips cotton swab test, a cough stress test, and a medium-fill, single-channel water cystometrogram. A normal residual urine result was defined as ≤ 50 ml. A positive urine culture was $\geq 10^5$ colonies per milliliter of urine. A mild cystocele was defined as the descent of the anterior vaginal wall to the midplane of the vagina while the patient was straining in the lithotomy position. A moderate cystocele was defined as the descent of the anterior vaginal wall through the midplane of the vagina but not beyond the introitus, and a severe cystocele was defined as the descent anterior vaginal wall outside the vaginal introitus. After the pelvic examination, a previously described Q-Tips cotton swab test was performed.[8] A maximum straining angle of ≥ 30 degrees from the horizontal was defined as a positive Q-Tips cotton swab test and was considered indicative of urethral hypermobility. The cough stress test was performed while the patient was in the standing position and had a symptomatically full bladder that contained at least 300 ml of fluid. The patient was asked to cough vigorously three times, and the test result was considered positive if leakage from the urethra was observed. A medium-fill, single-channel cystometrogram (Endotek OM-3 Max Digital Urodata monitor, Medical Engineering Corp., Racine, Wis.) was performed with a three-way 16F Foley catheter and saline solution at 38° C when the patient was standing. The patient was asked to cough and heel bounce at every 100 ml infused. Maximum bladder capacity and uninhibited detrusor contractions were observed. A detrusor contraction was defined as a sustained pressure rise of >15 cm H_2O while the patient inhibited voiding and did not execute Valsalva's maneuver.

At least 1 week after the initial evaluation, all patients underwent multichannel urodynamic testing with a six-channel recorder (Urolab model 1106, Life-Tech, Inc., Houston) and microtip pressure transducers (models PUPC-780 and PUPC-380, Life-Tech).[9] If a positive urine culture was found at the initial evaluation, urodynamic testing was delayed until a repeat culture showed resolution of the infection. Static and stress urethral pressure profiles were obtained when the patient was in both the supine and the sitting positions. Multichannel cystometry was performed when the patient was standing. Genuine stress incontinence was defined by pressure equalization on the stress urethral pressure profile in the absence of detrusor activity. Mixed incontinence was defined as genuine stress incontinence and detrusor instability in the same patient. All terms conform to the recommendations of the International Continence Society unless specifically stated.[10]

Demographic data, history, and clinical testing were correlated with the final urodynamic diagnoses. Independent variables were analyzed by either the χ^2 test or two-tailed Fisher's exact test, and $p < 0.05$ was considered statistically significant. The reproducibility of the predicted diagnoses by multichannel urodynamics was analyzed by the κ statistic.[11] A κ value of >0.75 denoted excellent reproducibility and κ values of ≥ 0.4 and ≤ 0.75 denoted good reproducibility. A κ value of <0.4 indicated poor reproducibility.

To define a meaningful combination of information that would allow the most accurate prediction of incontinence, we used a discriminant analysis program

Table II. Frequencies of answers to questions thought to discriminate diagnoses of incontinence

Question No.	Patients answering "yes" (%)			
	Genuine stress incontinence (n = 38)	*Mixed incontinence (n = 24)*	*Detrusor instability (n = 17)*	*Significance*
1. Stress incontinence with cough, sneeze, vomiting	94.7	100	70.6	$p = 0.003$
2. Stress incontinence with walking, running, etc.	100	91.7	52.9	$p < 0.001$
3. Ability to stop urine flow	57.9	41.7	52.9	NS
4. Large urine loss with cough, sneeze, etc.	29.0	50.0	41.2	NS
5. Urgency	23.7	37.5	58.8	$p = 0.041$
6. Urge incontinence	76.3	91.7	94.1	SN

NS, Not significant.

to determine the variables. When these variables were obtained, the most discriminating combination was determined and was used to predict the final diagnosis.

Results

The final diagnoses obtained by urodynamic testing are shown in Table I. No statistically significant association was found between age, parity, or race and the final diagnosis.

To analyze individual tests and data associated with the most common forms of incontinence, we further evaluated only those patients who had a final diagnosis of genuine stress incontinence, detrusor instability, or mixed incontinence.

Twenty-seven women studied had undergone a hysterectomy. There was no significant association between previous hysterectomy and incontinence. Previous surgery to correct incontinence had been performed in 26 patients. No statistically significant association was found between the type or number of procedures to correct incontinence and the final diagnosis of incontinence.

Responses to questions that distinguish genuine stress incontinence from detrusor instability are shown in Table II. The percentages of patients with genuine stress incontinence or mixed incontinence who answered "yes" to questions 1 and 2 were statistically different from those of patients with detrusor instability. However, of patients with only detrusor instability, 70.6% and 52.9% answered "yes" to questions 1 and 2, respectively. There was also a statistically significant difference in the incidence of urgency between patients with genuine stress incontinence and those with detrusor instability. The history of nocturia was not included in Table II because it was a continuous variable. No significant association was shown between the number of voids during the night and the final diagnosis.

Results of the six clinical tests and their associations with final urodynamic diagnoses are shown in Table III. The only tests that showed statistically significant associations were the cough stress test and single-channel standing cystometrogram. The cough stress test result was positive more frequently in patients with genuine stress incontinence or mixed incontinence, regardless of whether these groups were analyzed individually or together. It is of note that 17.7% of patients with detrusor instability demonstrated urine loss when they coughed. Uninhibited detrusor contractions during single-channel medium-fill cystometry were strongly associated with a final diagnosis of detrusor instability and/or mixed incontinence. However, 10.5% of patients with genuine stress incontinence had a positive screening cystometrogram. No statistically significant association was noted between the final diagnosis and a moderate to severe cystocele or urethral hypermobility.

The ability of a predicted diagnosis that is based on the cough stress test result and the single-channel cystometrogram to be reproduced by multichannel urodynamics is shown in Table IV. Good (but not excellent) reproducibility between predicted and final diagnoses was determined by κ value of 0.615. No patient with a predicted diagnosis of genuine stress incontinence was found to have only detrusor instability on urodynamic testing, but 17.9% did have mixed incontinence. No patient with a predicted diagnosis of detrusor instability was found to have genuine stress incontinence after urodynamic evaluation; however, 6.7% of those patients had mixed incontinence, and in another 13.3% no incontinence could be demonstrated. Urodynamic evaluation indicated that 50% of patients predicted as having no incontinence had etiologic factors for urine loss.

Discriminant analysis of the predictive data produced a function composed of six variables. The following six variables, in combination, were judged as discriminating: question 1, question 2, question 6, a history of one or more incontinence procedures, the cough stress test result, and the single-channel cystometrogram. In their most discriminating combination the best prediction of

Table III. Results of clinical tests in 79 patients undergoing multichannel urodynamic testing

Clinical test	*Final diagnostic groups with positive clinical test results (%)*			
	Genuine stress incontinence (n = 38)	*Mixed incontinence (n = 24)*	*Detrusor instability (n = 17)*	*Significance*
Urine culture >100,000 colonies/ml	5.3	8.3	5.9	NS
Moderate to severe cystocele	44.7	50.0	70.6	NS
Residual urine >50 ml	10.5	8.3	17.7	NS
Positive cough stress test result	89.5	91.7	17.7	$p < 0.001$
Positive Q-Tips cotton swab test result	84.2	91.7	76.5	NS
Evidence of detrusor instability on standing cystometrogram	10.5	70.8	88.2	$p < 0.001$

NS, Not significant.

Table IV. Reproducibility between predicted diagnosis, determined by cough stress test and single-channel cystometrogram, and final urodynamic diagnosis

Predicted diagnosis	*Final urodynamic diagnosis*							
	No incontinence (n = 8)		*Genuine stress incontinence (n = 38)*		*Mixed incontinence (n = 24)*		*Detrusor instability (n = 17)*	
	No.	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
No incontinence ($n = 10$)	5	50.0	3	30.0	0	0	2	20.0
Genuine stress incontinence ($n = 39$)	1	2.6	31	79.5	7	17.9	0	0
Mixed incontinence ($n = 23$)	0	0	4	17.4	16	69.6	3	13.0
Detrusor instability ($n = 15$)	2	13.3	0	0	1	6.7	12	80.0

A κ value of 0.615 indicates good reliability ($p < 0.001$).

genuine stress incontinence reached 89.5%. The best combinations of these variables that accurately predicted mixed incontinence or only detrusor instability reached 66.7% and 76.5%, respectively.

Comment

The aim of this study was to analyze the association of a patient's history and a group of predictive clinical tests with a final diagnosis of urinary incontinence determined by multichannel urodynamics. We hoped to provide the clinician with a practical and accurate method of evaluating patients with urinary incontinence and defining those patients who should undergo more extensive urodynamic testing. We found that a patient's history correlated poorly with the final diagnosis of incontinence. Only two clinical tests, the cough stress test and single-channel cystometrogram, provided reliable predictive information. Even so, reproducible prediction of urinary incontinence was not always possible with these two tests or a derived group of discriminant variables.

The patient's history has repeatedly been shown to be an unreliable predictor of the underlying cause of urinary incontinence.[1-6] Walters and Shields[3] and Cardozo and Stanton[1] showed a strong association between the symptom of stress incontinence and the final diagnosis of genuine stress incontinence. However, approximately 72% and 50%, respectively, of their patients with detrusor instability complained of stress incontinence. Our findings are very similar to those just cited. In addition, 76.3% of our patients with genuine stress incontinence related a history of urge incontinence. The patient's history must be highly sensitive and specific to be a reliable diagnostic tool. We have shown poor specificity of the history in predicting the cause of urinary incontinence. The patient's history is not useful diagnostically as a result of the high degree of overlap in findings among the various forms of incontinence. Obviously, the major fear of using the history to diagnose incontinence is that unnecessary surgery will be performed. Rather, the patient's history should be used to guide the subsequent evaluation process and to serve as a measure of the patient's disability.[1]

We found that many commonly performed objective clinical tests are of little diagnostic value. A urinary tract infection or a residual urine value of >50 ml was not associated with any particular diagnosis. However, these tests should always be a part of the evaluation of a woman who has lower-urinary tract symptoms. Investigation in the presence of a urinary tract infection, especially with invasive techniques, could worsen a patient's condition. A residual urine value can contribute

to the investigation of overflow incontinence and may be predictive of postoperative voiding difficulty, although our data are insufficient to support this. A moderate to severe cystocele and the Q-Tips cotton swab test, which are the clinical measures of anatomy, were not predictive of incontinence. Although a large cystocele has been associated with genuine stress incontinence, we found that it occurred more commonly in our patients with detrusor instability, although this occurrence was not statistically significant. In addition, there was no significant difference in the incidence of urethral hypermobility among any of the diagnostic groups. However, when the Q-Tips cotton swab test was originally described, its function was never diagnostic.[12] Rather, recent data support its value as a predictor of success with surgery to correct incontinence.[8, 13]

The cough stress test and standing single-channel medium-fill cystometrogram were the only clinical tests statistically associated with final urodynamic diagnoses. This is supported by the findings of Fischer-Rasmussen et al.,[6] who found that the cough stress test and cystometrogram were the most predictive of a battery of tests used to diagnose urinary incontinence. In spite of the statistical significance that was demonstrated in our study, the overlap in positive results brings the clinical ability to distinguish diagnoses into question. In 18% of patients with detrusor instability a cough stress test result was positive, and almost 10% of patients in the groups with genuine stress incontinence and mixed incontinence did not have leakage when they coughed. Again, the danger of basing the diagnosis on this test alone is that its results may indicate unnecessary surgery. Performing cystometrography most certainly reduces this potential. However, the single-channel cystometrogram is subject to false-positive results, and cystometry may not indicate the diagnosis of detrusor instability.[14] Repetitive testing with single-channel cystometry improves diagnostic accuracy.[15]

When we used the cough stress test and single-channel cystometrogram together to make a prediction of incontinence, we reduced the potential for inappropriate surgery: No patient with a predicted diagnosis of genuine stress incontinence had a diagnosis of detrusor instability after urodynamic testing. However, the overall reproducibility of our predicted diagnoses was limited. When we used a combination of variables obtained by discriminant analysis, we achieved no improvement in the reliability of predicting a diagnosis of incontinence. One assumption made during this study was that multichannel urodynamics always provided a correct diagnosis. To assume that urodynamic testing is infallible is naive. Constraints set by a laboratory environment may not allow the demonstration of urine loss that occurs when a patient is uninhibited in her own home.[6] This can result in false-negative diagnoses, especially that of detrusor instability.[14] A diagnosis of genuine stress incontinence that is based on urethral pressure profilometry has been questioned.[16, 17] Richardson[16] suggested that excessive urethral hypermobility may result in false pressure equalization during coughing. However, in most instances genuine stress incontinence may be validated with a stress urethral pressure profile.[9, 18] In our group of limited diagnostic techniques, multichannel urodynamics remains a valuable and accurate tool.

We have shown that the patient's history alone is unreliable in making a diagnosis of urinary incontinence. The use of specific office tests improves the reliability of a predicted diagnosis. However, when almost complete accuracy is necessary to avoid inappropriate therapy, it is our opinion that patients with complaints of urinary incontinence should, when available, undergo multichannel urodynamic testing. This is extremely important when surgery is contemplated.

Alternatively, for patients in whom the cough stress test is negative and a single-channel cystometrogram demonstrates detrusor instability, an initial trial of medical or behavioral therapy is acceptable. Urodynamic testing may be performed later if the patient fails to show improvement.

For the clinician who does not have the technical assistance of multichannel urodynamic testing, the office evaluation process that we have described, especially with a cough stress test and cystometry, should be mandatory. Improvement in urodynamic techniques is still needed and may be supplemented by videocystourethrography and pad testing. Further investigation is necessary.

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Discussion

Dr. Jeffrey L. Cornella, Scottsdale, Arizona. The correlation of diagnosis made by sophisticated urodynamic studies versus that made by office evaluation is worthy of continued study. Inappropriate diagnosis or unnecessary testing is costly in several respects. The objective of testing is to avoid incorrect patient treatment. This study correlated multichannel urodynamic testing with symptom survey and office testing. The authors may be commended for a well-designed study and statistical analysis, yet several problems exist in making these comparisons with our present technology.

The authors recognized that urethral profilometry and dynamic pressure equalization may not be considered a gold standard in these comparisons. If one compares two inaccurate tests, how are the results to be interpreted? This study defines stress incontinence in the advanced testing group as dynamic pressure equalization in the absence of detrusor contraction. This may induce false-positive results. As Richardson[1] has noted, a telescoping urethra when the patient is straining may move relative to the microtransducer.

Select patients who have otherwise normal urethras but who demonstrate descent with stress incontinence benefit from routine operation. Current understanding of pressure transmission ratios, which are directly related to dynamic equalization, indicates the usefulness of these ratios in predicting the degree of surgical success postoperatively, but they cannot be used to differentiate preoperative leak versus nonleak profiles.[2] Diagnosis should be limited to those who have overt urine loss in the absence of a contracting detrusor.

In addition, in patients with detrusor instability the diagnosis may not be manifest on a given cystometrogram. Ambulatory testing may improve the percentage of patients who receive an accurate diagnosis. Continuous ambulatory monitoring studies often reveal a higher incidence of unstable bladder contractions than do isolated cystometrograms.[3]

This article follows the standard International Continence Society's definition of the requirement of a 15 cm H_2O contraction for the diagnosis of detrusor instability. When detrusor instability is compared with symptoms, 15 cm H_2O has been demonstrated to be artificial. Coolsaet et al.[4] investigated women with bladder instability and/or stress incontinence. Subthreshold instability was noted to correlate with the symptoms of urgency. Eighty-seven of 334 patients studied had subthreshold contractions. Only 7% of this population had an asymptomatic history of freedom from urgency incontinence and frequency of urgency sensation. The correlation of testing results and symptoms was much higher in these studies.

Venrooij et al.[5] have also noted a difference in the diagnosis of detrusor instability made by diuresis cystometry in comparison with that made by retrograde filling. Higher levels of instability were noted with diuresis filling, which suggests a relationship to antecedent flow through the ureter that aids the demonstration of detrusor instability.

The questions in this survey were similar yet different from those asked by Walters and Shields.[6] Future surveys with multiple discriminatory questions may be more predictive and may establish surveys that can be used in subsequent studies. Few surveys have been studied objectively. Future studies may establish the use of history as a predictor of diagnosis to a greater extent.

It was noted that 88.2% of patients with a final diagnosis of detrusor instability had a positive test result from a standing cystometrogram. In a previous study by the Long Beach group[7] that compared the standing cystometrogram with multichannel urodynamic testing, it was found that only 59.3% of those with a final diagnosis of detrusor instability on multichannel testing had detrusor instability on standing single-channel cystometry. These results, which use the same methods in the same institution, vary.

In addition, it has been demonstrated that patients who have severe cystocele may have kinking of the urethra, which prevents demonstration of stress urinary incontinence. As the prolapse is reduced, the patient manifests this diagnosis. This has been demonstrated by Richardson et al.[8] and by other investigators. The number of patients with severe cystocele was not noted in the article and it was not evident whether these patients had a reduction of the cystocele at any point during testing for possible stress incontinence. Perhaps some of these patients had urine loss at home from coughing, laughing, or sneezing as the prolapse receded; this may not have been noted on multichannel testing without manual reduction.

The statistical analysis in this article appears to be very appropriate. However, the origin of the statement

"The prediction of genuine stress incontinence reached 89.5%" is unclear. This article also states that 17.6% of patients with a final diagnosis of detrusor instability had urine loss when they were coughing, which could be a secondary effect of a concomitant contraction. It would be interesting to know the correlation of detrusor instability on multichannel testing in this group. If this correlation is not high, it also reflects the difficulty of making these determinations.

It is my impression that this article is an important contribution to the body of investigation on the question of the reliability of patient history and various testing modalities in the diagnosis of urinary incontinence. Thorough and sophisticated testing remains an essential component of patient evaluation. However, it is also my impression that we cannot conclude that office evaluation and history do not have significant predictability of diagnosis. Additional studies are needed as we increase our understanding and advance urodynamic technology.

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DR. LANE J. MERCER, Chicago, Illinois. It is often difficult for a practicing physician to determine the standard of care for making a diagnosis or performing surgery. This is especially true in the preoperative evaluation of a patient with urinary incontinence. Although multichannel urodynamic testing has been shown to be the most reliable preoperative predictor of successful postoperative surgical correction of stress urinary incontinence, this technique remains expensive and is not readily available to all practitioners. Indeed, many experienced clinicians state that they can diagnose genuine stress incontinence from the patient's history and physical examination. With these diagnostic methods they can limit the referrals for multichannel urodynamic evaluation and can save the patient an invasive procedure, reduce medical costs, and increase patient convenience. However, these anecdotal data have not been supported by studies.

In 1978 Dr. Stuart Stanton stated, "In order to make the correct diagnosis (of stress incontinence) the clinician must rely on a thorough history and clinical examination aided by shrewd selection of the appropriate investigations." However, Dr. Stanton was unable to delineate exactly which were the appropriate investigations to be performed. Several authors attempted to outline more clearly the appropriate history and procedures necessary to ensure the accuracy of the diagnosis of incontinence: In 1975 Farrar et al.[2] reported that 96.4% of patients with a history of stress incontinence had stable bladders by urodynamic evaluation but that 89% of those who complained of urge incontinence had evidence of unstable bladders. Later work did not support this. In 1980 Cardozo and Stanton[3] showed that only 38% of women with detrusor instability reported urge and that nearly half of the women who complained of stress incontinence had unstable bladders; this proves that the patient's history was highly unreliable as diagnostic information.

In 1982 Kauppilla et al.[4] reported a detrusor instability score that used multiple questions thought to be relevant to the diagnosis of detrusor instability. The higher the score, the more likely the presence of detrusor instability and the less likely that surgical treatment could correct the incontinence. Indeed, those authors were able to predict 90% of the successful surgical corrections.

In 1988 Sand et al.[5] found that only 86.9% of the patients who complained of stress incontinence had genuine stress incontinence, although 36.6% who complained of urge or urge incontinence had detrusor instability. In the same year Walters and Shields[6] used history and the physical finding of a moderate to large cystocele to achieve a 100% sensitivity for genuine stress incontinence. However, the specificity was only approximately 22%. However, this ruled out 96% of other urologic disorders.

In their article the authors considered many historic and objective criteria to determine their correlation with the ultimate diagnosis of urinary incontinence made by multichannel urodynamic testing. Indeed, the ability to correlate these commonly and easily performed outpatient test results and history questionnaires with urodynamic evaluation was only poorly to moderately successful. However, they put forth a number of questions that may help clarify that correlation and may make it more clinically useful.

In this study surgery to correct incontinence had been performed in 26 patients. What type of surgery was performed in these patients, and did the type of surgery correlate with the type of incontinence found later? As most will agree, patients with recurrent incontinence or with complex histories of incontinence deserve multichannel urodynamic evaluation. Is it appropriate to include these patients in this study, which is more applicable to primary incontinence? Although no statistical difference was found between the individual diagnosis and the patient's age, it is well recognized that older women more frequently have uncontrolled bladder contractions, the relevance of which is

unknown. Therefore was there a statistical correlation among the age of the patient, the presence of uncontrolled bladder contractions, and the diagnosis of a mixed disorder?

In the patients studied more than a week passed between the initial testing and subsequwnt multichannel urodynamic testing. Detrusor instability is known to be intermittent and is perhaps suppressed or provoked by the anxiety of the first test. How much time elapsed between the first testing and the subsequent full urodynamic evaluation, and do the authors think that this may be relevant to the poor predictive value for detrusor instability.

The authors place equal weight on all the tests and questions presented. Would a weighted scale or a logistic regression analysis be more appropriate in the development of a formula to predict the final urodynamic diagnosis? Also, there are no obvious internal controls in the questionnaire to ensure adequacy or reproducibility of the patients' responses to the questions. Would not such an internal control be more useful with historic data?

Finally, as a result of this early work, what is recommended as the appropriate preoperative evaluation for a physician in a clinical setting who has a patient presenting with symptoms consistent with stress incontinence and findings of anterior vaginal wall prolapse?

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DR. SUSAN R. JOHNSON, Iowa City, Iowa. I think that this type of technology assessment is important. Several of the participants in the discussion mentioned cost, and I wonder if the authors have taken their data one step further and looked at a cost-benefit analysis that would compare the outcomes of those who undergo surgery and those who do not as a result of this 90% predictive ability?

This would involve, for example, comparing the cost of performing only simple tests in 100 women, which would result in 10 unnecessary operations versus the cost of performing multichannel testing in all 100 women, which would eliminate those 10 unnecessary operations by means of testing that is more sophisticated.

DR. JOHN O.L. DELANCEY, Ann Arbor, Michigan. I would like to raise a philosophic question. The underlying assumption of many laboratory evaluations of urinary incontinence is that the cystometrogram reliably detects detrusor instability. As Dr. Cornella mentioned, we have realized that this is not at all the case.

A physical examination can be used to evaluate stress urinary incontinence. The entire framework of determining whether a patient has mixed incontinence or detrusor instability tests on the accuracy of the cystometrogram in the detection of detrusor instability. I wonder whether we shouldn't examine the fundamental assumption that a cystometrogram is an accurate diagnostic test.

A diagnostic error can be made if a 10-minute cystometrogram is used to exclude the diagnosis of stress urinary incontinence in an intelligent woman who describes an episode of detrusor instability and who has a negative cystometrogram.

DR. MICKEY KARRAM, Cincinnati, Ohio. I just want to reemphasize the point that discussants are making about detrusor instability.

The most recent International Continence Society recommendations and definitions no longer use a cutoff of 15 cm H_2O to define detrusor instability. The current definition is any increase in true detrusor pressure that is believed not to result from bladder compliance. A significant number of patients will have symptomatic urgency and urge incontinence but will not have rises of 15 cm H_2O. I am wondering why the current and most recent definition was not used in this study.

The second point concerns the use of a cutoff level of 30 degrees to define a positive or negative Q-Tips cotton swab test. No data in the literature support this, and I think that it is much better to describe bladder neck mobility by simply stating the straining angle.

Finally, the patients who had had previous surgery and recurrent stress incontinence are a separate group because the clinician must define or identify their condition and must also determine the type of stress incontinence (anatomic or sphincteric). Could the authors comment on this?

DR. JOSEPH C. SCOTT, JR., Omaha, Nebraska. I am curious about the degree-of anatomic defects in this patient population. I know that the authors are probably limited as to the information they can present, but it would be helpful if they could comment on the severity of the anatomic defects in this group of patients.

DR. SUMMITT (Closing). I knew that this topic would generate a significant amount of discussion. Whether I am equal to my discussants or questioners will be seen at the end. I shall do my best.

I shall begin my closing discussion in the order of questions received. Let me first address the question of urethral pressure profilometry and specifically some of the inaccuracies that have been suggested. I hope that I emphasized that this is not a "definitive diagnostic method." With regard to Dr. Cornella's question about

two different, inaccurate methods, we are limited by the technology we have. We use the technology available to us within diagnostic limitations, and we compensate for some of the inaccuracies by the use of direct observations.

In our methods we considered the study by Richardson (see reference 16 of the article), which showed a telescoping effect of the urethra that caused false positivity in pressure equalization during the cough profile. We do attempt to eliminate the deviation in pressure that occurs with movement. However, that is not always consistently possible, and we realize the intrinsic problems with that technique.

There also are problems when the patient does not cough hard enough. The patient may actually have stress incontinence that is not identified with cough urethral pressure profilometry. We attempt to repeat cough profiles several times during the same testing period to compensate for this inaccuracy.

Now I will address the use of specific definitions in our study. Because primarily one investigator performed the testing, we used rigid definitions of stress incontinence both for office testing and in urodynamic testing to avoid bias. I realized from the beginning that this might be a problem in the article, but we avoided the biases that might have arisen by our knowledge of the patient from earlier testing.

With reference to the validity of the patient's history, there are few data that show a good correlation of history with the final diagnosis of incontinence. Patient history can often be misleading. I think that the history is valuable as a guide to the evaluation. What brings the patient to your office is her complaint of urinary incontinence. Use the patient's history as a guide to and a measure of the severity of incontinence.

Now I would like to address the discrepancy in the cystometric findings described (88.5% accuracy with single-channel cystometrics) as opposed to the cystometric results (55%) in an earlier study by Sand et al. at the same institution.

The earlier study was a review in which both supine and standing cystometry were examined. Our study was a prospective evaluation with a single examiner who performed standing cystometry according to specific definitions and who correlated the cystometric results with the final diagnoses for which one of the other investigators and I had performed the multichannel urodynamic studies. It is my feeling that our improved methods probably account for the discrepancy noted.

Finally, I want to say that I am not discouraging the use of office testing, but I think that it is important to remember that even when the patient's history and office testing are used to make diagnoses there is a 10% to 15% inaccuracy rate in treatment that is based on these preliminary test results. This has been well documented in the article by Walters and Shields. (Dr. Cornella's reference 6).

In answer to Dr. Mercer's questions about previous surgery, we did review the numbers of operations, which ranged from one to four. There was no statistical correlation between the numbers of previous operations and the final diagnoses of incontinence. We even considered the types of operations. The majority of our patients had simple anterior repairs; the second highest number were retropubic urethropexy (primarily Marshall-Marchetti-Krantz) procedures. There was no statistical association between the type of prior incontinence surgery and the final diagnosis.

We did consider the patient's age, although I did not include that in the article. There was no statistical correlation between age and the final diagnosis.

Are contractions clinically relevant in the group with mixed incontinence? I think so. There is no consensus about treating patients with mixed incontinence, although most recommendations concern treating detrusor instability first. If involuntary contractions improve, the need for surgery can be reassessed. Treating detrusor instability may relieve the patient's incontinence so that she may desire no further therapy. In reference to Dr. Mercer's question about multivariant analysis, we did not use that method specifically. That has been done by Fischer-Rasmussen et al. (see reference 6 of article), who found no statistical correlation between that method and the final diagnosis.

In answer to the question of what to do for those patients for whom urodynamic testing is not available, I still think that according to our data, the patient's history is initially valuable in guiding the evaluation because it delineates the patient's problems and their severity. In the next step, which is clinical office testing, the Q-Tips cotton swab test has been valuable, not as a diagnostic tool but as a prognostic factor for surgery in certain patients. Several studies in the last 5 years showed that patients who have minimal mobility of the urethra and who also have stress incontinence may be at greater risk for failure with standard surgery to correct incontinence. That may partially address Dr. Karram's questions. I recommend that cystometrography be performed in addition to a cough stress test. The American College of Obstetricians and Gynecologists quality assurance criteria for performing surgery in patients recommend that a cystometrogram be obtained and that objective demonstration of urine loss be seen. This battery of tests should be the minimum evaluation when urodynamic testing is unavailable.

Now I would like to address the last questions. Dr. Johnson asked about the cost-benefit analysis. We have not examined this. Around the country, costs for multichannel urodynamic testing range from $200 to $400. Office testing costs can range up to $150 for a cystometrogram. I have not compared these tests, but that would be an interesting analysis.

With respect to Dr. DeLancey's questions about the cystometrogram, I think that most clinicians who perform urodynamic testing realize the minimal inaccuracies of cystometry. We realize that what may occur during 24 hours in a patient's life may not occur during a 10-minute test in the office. Sand et al. examined

simple office cystometry and suggested that repetitive testing on several occasions may result in a diagnosis of detrusor instability. We are considering that in further investigations.

With regard to Dr. Karram's question about the new definition of detrusor instability, we began this study in 1987, and we used the current definition at that time. As I said, we tried to maintain specific definitions throughout the study period to avoid bias in patient evaluation.

As far as the 30-degree cutoff for the Q-Tips cotton swab test, we tried to maintain specific definitions. In our data analysis we did not feel as if we could accurately use various measures of angle deviation in the Q-Tips cotton swab test. We maintained the 30-degree definition for ease of data analysis.

In answer to Dr. Scott's question about the degree of anatomic defects, we again made specific cutoffs, which may be a problem in the article with respect to the analysis of the patient population. The nature of our patient population may have skewed the anatomic findings. The majority of our patients did not have severe cystoceles or significant Q-Tips cotton swab test deviations.

Mechanism of recurrent spontaneous abortion

I. Ultrasonographic findings

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OBJECTIVES: Our objective was to study the mechanisms of early pregnancy loss with ultrasonographic findings in pregnancies that terminated in repeat abortion in women with recurrent spontaneous abortion compared with patients with spontaneous abortion with no previous spontaneous abortion and with women with recurrent spontaneous abortion who had viable pregnancies.
STUDY DESIGN: A prospective, unselected study of 83 pregnancies followed from 4 to 12 weeks of gestation. Weekly transvaginal ultrasonography among 43 pregnancies terminating in abortion were compared with 40 pregnancies ending in live birth with the Fisher exact test with a p value of significance assigned at <0.05.
RESULT: A significant difference in the presence of fetal heart activity was observed at 6 weeks ± 5 days among women who subsequently aborted when recurrent spontaneous abortions were compared with no history of abortion (55% and 14%, $p < 0.02$). Fetal pole size was small in 86% of pregnancies lost after fetal heart activity.
CONCLUSION: Ultrasonographic examination at 6 weeks can diagnose impending abortion with a sensitivity of 85%, specificity of 93%, positive predictive value of 92%, and negative predictive value of 87% among women with recurrent spontaneous abortion. (Am J Obstet Gynecol 1992;166:1844-52.)

Key words: Recurrent spontaneous abortion, ultrasonography

The inability of a couple to achieve desired family size affects an estimated three million couples in the United States.[1] Reproductive life-table analysis indicates that the majority of reproductive failures results from postfertilization failures occurring before or after implantation.[2] Childlessness as a consequence of repeated postimplantation losses has been described as a syndrome[3] termed recurrent spontaneous abortion. Although the syndrome has been defined as three or more consecutive spontaneous abortions,[3] a number of studies include those with two consecutive spontaneous abortions. Rationale for including women with two spontaneous abortions is provided by the observations that the greatest change in risk of losing a subsequent pregnancy comes after two consecutive abortions,[4] and no differences in the prevalence of etiologic factors exist between couples with three or more abortions.[5]

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6/6/37124

Table I. Summary of ultrasonographic findings during the first trimester of 83 pregnancies observed in 69 women with a history of recurrent spontaneous abortion and in 14 women with no previous history of abortion

Ultrasonographic finding	*RSA (n = 40)*		*RSA (n = 29)*		*Non Ab (n = 14)*		*Total Ab (n = 43)*	
	No.	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
No gestational sac	0	0	5	17	2	14	7	16
Empty sac	0	0	8	28	4	29	12	28
Fetal pole without fetal heart activity	0	0	2	7	1	7	3	7
Fetal heart activity	40	100	14	48	7	50	21	49

ASA, Recurrent spontaneous abortion; *Non Ab,* no history of abortion.

Recurrent spontaneous abortion occurs in 2% to 5% of reproductive aged women each year.[4, 6] The etiologies have been reported as being due to anatomic, hormonal, chromosomal, and immunologic causes.[7] Although recent reports suggest that immunologic causes account for a number of heretofore unexplained cases,[8-11] the mechanisms of fetal loss are not known. To study the mechanisms of early pregnancy loss, 83 pregnancies were monitored ultrasonographically during the first 12 weeks of gestation. This report describes the ultrasonographic findings of pregnancies occurring in women with recurrent spontaneous abortion that terminated in repeat abortion and compares these findings with those of spontaneous abortions occurring in women with no previous spontaneous abortions and in women with recurrent spontaneous abortion who had viable pregnancies.

Material and methods

Pregnancies. A total of 83 pregnancies were studied (January 1990 to December 1990) from week 4 to week 12 of gestation. Of these 83 pregnancies, 43 terminated in first trimester spontaneous abortions and 40 produced live births. A previous history of recurrent spontaneous abortion was present in 69 pregnancies, and 14 pregnancies occurred in women with no previous abortions. Among the 69 pregnancies occurring in women with a history of recurrent spontaneous abortion, 40 resulted in a viable birth and 29 ended in repeat abortion. An abnormal chromosome analysis was documented in seven of the products of conception from first trimester spontaneous abortions.

Ultrasonographic monitoring. Eighty-three pregnancies were followed during the first 12 weeks of gestation with an ATL Ultramark ultrasonography machine with a 5 MHz transvaginal probe (Advanced Technology Laboratories, Bothell, Wash.). At the time of each examination the gestational sac and yolk sac were measured in three diameters, and these measurements were averaged and recorded. The length of the fetal pole was measured and recorded. The presence or absence of fetal heart activity was noted. The size and rate of growth of the gestational sac and fetal pole were compared with those expected from the last menstrual period (LMP), as determined by the method of Hellman et al.[12] and Silva et al.[13]

Progesterone and human chorionic gonadotropin determinations. At the time of each ultrasonographic examination, blood was drawn from the antecubital vein of each woman for determination of progesterone and human chorionic gonadotropin (hCG). These levels were determined by radioimmunoassay (Diagnostic Products, Los Angeles). Statistical analysis was performed with Fisher's exact test with a p value of significance assigned at <0.05.

Results

The overall ultrasonographic findings during the first trimester observed in 83 pregnancies are summarized in Table I. All 40 pregnancies producing a live birth demonstrated a gestational sac containing a fetal pole with fetal heart beat visualized on real time. Among the 43 pregnancies terminating in abortion, 16% displayed no gestational sac, 28% revealed a gestational sac with no discernible fetal pole, 7% had a fetal pole with no observable heartbeat, and 49% demonstrated the presence of fetal heartbeat. No differences in the frequencies ultrasonographic findings were observed when pregnancies in women with a history of recurrent spontaneous abortion were compared with no history of previous abortion. In addition, no differences in the frequency of the presence or size of the yolk sac were seen when pregnancies in women with a history of recurrent spontaneous abortion were compared with a history of no previous abortion.

Seven of the abortuses displayed an abnormal chromosome analysis. Two chromosome abnormalities were found in patients with history of recurrent spontaneous abortion (47,XX +15, 48,XX +13 +16) and five in those patients with no prior abortion (69,XX, 47,XX +22, 45,X, 47,XX +22, 69,XXX). The abnormal karyotypes observed are listed in Table II and the ul-

Table II. Chromosome analysis among seven abortuses displaying abnormal karyotypes

Abnormal karyotype	*No.*	*Obstetric diagnosis*
47,XX+22	2	Non AB
47,XX+15	1	RSA
48,XX+13+16	1	RSA
69,XXX	2	Non AB
45,X	1	Non AB

Non AB, No history of previous abortion; *RSA,* history of recurrent spontaneous abortion.

Table III. Ultrasonographic findings during first trimester in pregnancies terminating in abortion in which abnormal karyotype was found

	Pregnancies	
Ultrasonographic findings	*No.*	*%*
No gestational sac	0	0
Empty sac	0	0
Fetal pole without fetal heart activity	1	14
Fetal heart activity	6	86

trasonographic findings in these pregnancies are shown in Table III. All pregnancies demonstrating an abnormal karyotype had a fetal pole visible on ultrasonographic examination, and all but one displayed the presence of fetal heart activity.

The frequency of ultrasonographic findings at specific weeks of gestation during the first trimester among women with a history of recurrent spontaneous abortion are shown in Table IV. All pregnancies revealing no gestational sacs were diagnosed by 6 weeks of gestation. Also by 6 weeks of gestation 95% of all pregnancies producing a live birth demonstrated the presence of fetal heart activity. Table V summarizes the findings of a single ultrasonographic examination performed at 6 weeks ± 5 days in successful and unsuccessful pregnancies among women with a history of recurrent spontaneous abortion and no history of previous abortion. A significant difference in the frequency of finding the presence of fetal heart activity was observed in pregnancies producing a live birth compared with pregnancies ending in abortion ($p < 0.00001$) and in those pregnancies terminating in abortion when women with a history of recurrent spontaneous abortion were compared with women with no previous history of abortion ($p < 0.01$). Among 29 pregnancies terminating in abortion in women with a history of recurrent spontaneous abortion, 16 (55%) demonstrated the presence of fetal heart activity on an ultrasonographic scan performed at 6 weeks' gestation. By contrast, two of 14 (14%) of pregnancies terminating in abortion among women with no previous abortion displayed fetal heart activity at 6 weeks of gestation.

Table VI describes the size of the fetal pole compared with the expected size on the basis of the LMP among those pregnancies demonstrating fetal heart activity at 6 weeks' gestation. The majority (86%) of pregnancies that aborted after the establishment of fetal heart activity had fetal poles that were <0.6 week from expected size on the basis of the LMP. By contrast, only 10% of pregnancies ending in live birth had fetal poles measuring <0.6 weeks from expected size at 6 weeks' of gestation. Serum progesterone and hCG concentrations were not low among those pregnancies terminating in abortion after demonstration of fetal heart activity at 6 weeks (Table VII).

Serum progesterone and hCG concentrations in pregnancies from weeks 4 through 12 of gestation among 69 women with a history of recurrent spontaneous abortion are summarized in Tables VII and VIII. No differences in progesterone or hCG concentrations were observed when these values were compared among women aborting their pregnancies and those producing a live birth. Table IX describes those pregnancies in which serum progesterone concentrations drawn at 6 weeks' gestation were <10 ng/ml. Fourteen of the 83 pregnancies (17%) studied produced serum concentrations of progesterone concentrations of <10 ng/ml at 6 weeks' gestation; nine of these 14 pregnancies (64%) occurred in women who subsequently aborted and five (36%) were observed in women who delivered a live birth. Pregnancies terminating in abortion had progesterone levels of <10 ng/ml at 6 weeks 21% of the time. When those 14 pregnancies with low progesterone concentrations at 6 weeks were correlated with ultrasonographic findings at the same period of gestation, a difference between pregnancies ending in live birth and abortion was seen (Table X). All pregnancies associated with progesterone concentrations of <10 ng/ml that subsequently ended in live birth had fetal heart activity on ultrasonographic examination at 6 weeks, and 80% of these demonstrated sizes of fetal poles consistent with expected dates. In contrast, one of the nine pregnancies (11%) with low progesterone values that subsequently terminated in abortion had fetal heart activity documented on ultrasonographic examination. This one pregnancy had a fetal pole smaller than expected size. The remaining 89% of pregnancies with low progesterone concentrations displayed an empty sac or a small fetal pole with no demonstrable cardiac activity at the time the low progesterone values were observed.

Comment

The ultrasonographic observations in this study help to describe mechanisms involved in spontaneous abortion. No gestational sac was observed in 16% of preg-

Table IV. Ultrasonographic findings of 69 pregnancies from weeks 4 to 12 to gestation among women with a history of recurrent spontaneous abortion that terminated in abortion compared with those producing a live birth

	Weeks' gestation (% pregnancies)							
	4		*5*		*6*		*>7*	
Ultrasonographic finding	*Abortion*	*Live birth*	*Abortion*	*Live birth*	*Abortion*	*Live birth*	*Abortion*	*Live birth*
No gestational sac	27	63	17	8	0	0	0	0
Empty sac	73	37	83	38	6	5	16	3
Fetal pole without fetal heart activity	0	0	0	18	38	0	26	2
Fetal heart activity	0	0	0	36	56	95	58	95

Table V. Ultrasonographic findings at 6 weeks' gestation in 83 pregnancies among 69 women with a history of recurrent spontaneous abortion and 14 women with no previous history of abortion

	RSA (n = 40)		*RSA (n = 29)*		*Non Ab (n = 14)*		*Total Ab (n = 43)*	
Ultrasonographic finding	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
No gestational sac	0	0	0	0	0	0	0	0
Empty sac	2	5	1	3	6	43	7	16
Fetal pole without fetal heart activity	0	0	12	42	6	43	18	42
Fetal heart activity	38	95*	16	55†	2	14†	18	42*

RSA, Recurrent spontaneous abortion; *Non Ab,* no history of abortion.
*Difference is significant ($p < 0.00001$).
†Difference is significant ($p < 0.01$).

nancies terminating in abortion, empty gestational sac in 28%, and fetal pole with absence of fetal heart activity in 7%. In 49% of spontaneous abortions, the pregnancy was lost after demonstration of fetal heart activity. The frequencies of these ultrasonographic findings were significantly different when abortions in women with a history of recurrent spontaneous abortion were compared with those in women with a history of no previous abortions at 6 weeks' gestation. Fifty-five percent of women with a history of recurrent spontaneous abortion aborted after documentation of fetal heart activity at 6 weeks, whereas 14% of women with no history of previous abortion demonstrated the presence of fetal heart activity at 6 weeks' gestation.

Among all pregnancies that aborted after demonstration of fetal heart activity, the ultrasonographic date of the pregnancy determined by measurement of the fetal pole was <0.6 week from expected dates on the basis of the LMP in 86%, suggesting that blood flow to the embryo was diminished. Immunohistochemical findings from placentas of first-trimester spontaneous abortions support this view in that 50% of conceptuses that were aborted have evidence of an increase in immunopathology.[14] This lesion is associated with activated endothelial cells of fetal stem vessels. Thrombomodulin disappears from plasma membranes of activated endothelial cells and tissue factor appears.[15] Tissue factor activates factor VII of the extrinsic coagulation pathway, leading to fibrin deposition and platelet-fibrin thrombi.[16] Thus the fetal stem vessel endothelial surface is changed from a thromboresistant surface to a thrombogenic surface, and blood flow to the growing conceptus is diminished. Khong et al.[17] described changes in spiral arteries in placental beds from abortuses. The physiologic changes occurring in the spiral arteries from weeks 6 through 12 of gestation that result in increased blood flow through the uteroplacental arteries and into the intervillous space were absent.[18] Physiologic changes are the result of migration of extravillous trophoblasts from the cytotrophoblastic shell and tips of anchoring villi into decidua basalis and subsequent myometrium and migration of endovascular trophoblasts retrogradely into the walls of spi-

Table VI. Comparison of size of fetal pole with expected size on basis of LMP among pregnancies demonstrating fetal heart activity

	Pregnancy outcome			
	Viable (n = 38)		*Abortions (n = 18)*	
Size expected from LMP	*No.*	*%*	*No.*	*%*
≥0.6 wk	34	90	3	14
<0.6 wk	4	10	15	86

Table VII. Progesterone concentrations in pregnancies from weeks 4 through 12 of gestation among 69 women with history of recurrent spontaneous abortion that terminated in abortion compared with those producing live births

	Weeks' gestation (%)							
	4		*5*		*6*		*>7*	
Progesterone (ng/ml)	*Abortion*	*Live birth*	*Abortion*	*Live birth*	*Abortion*	*Live birth*	*Abortion*	*Live birth*
0-10	8	12	13	23	17	25	16	38
11-20	50	33	41	23	42	37	36	46
21-30	19	0	24	15	24	13	31	8
31-40	8	22	13	31	5	25	10	8
≥41	15	33	9	8	12	0	7	0

Table VIII. hCG concentrations in pregnancies from weeks 4 through 12 of gestation among 69 women with history of recurrent spontaneous abortion that terminated in abortion compared with those producing live births

	Weeks' gestation (%)							
	4		*5*		*6*		*>7*	
hCG (mIU/ml)	*Abortion*	*Live birth*	*Abortion*	*Live birth*	*Abortion*	*Live birth*	*Abortion*	*Live birth*
0-10,000	83	80	51	50	27	30	11	31
11,000-20,000	7	20	22	25	23	30	23	25
21,000-30,000	3	0	7	8	2	20	4	12
31,000-40,000	0	0	10	0	11	20	13	6
≥41,000	7	0	10	17	37	0	49	26

Table IX. Pregnancy outcome in which serum progesterone concentrations at 6 weeks' gestation were <10 ng/ml

Pregnancy outcome	*No. of pregnancies*	*Progesterone <10 ng/ml*	
		No.	*%*
Viable	40	5	13
Abortion	43	9	21
Recurrent spontaneous abortion	29	6	21
No history of abortion	14	3	21
TOTAL	83	14	17

ral arteries. The muscular and elastic tissue in the arterial wall are replaced by a fibrinoid material converting them into flaccid distended tubes, allowing a tenfold increase in blood flow. Any event that would diminish blood flow to the fetus through spiral arteries or fetal stem vessels would result in fetal starvation. The ultrasonographic observations of pregnancy loss after establishment of fetal heart activity of smaller-than-expected fetal poles would suggest a problem in fetal blood flow. Because physiologic changes in the spiral artery do not occur until 6 weeks of gestation and because the fetal pole is already smaller than the expected size by 6 weeks of gestation, the compromise in fetal blood flow most likely occurs within the fetal stem vessels. Evidence of fetal starvation before abortion occurs in 88% of pregnancies from women with a history of recurrent spontaneous abortion.

That the mechanism of abortion after the establishment of fetal heart activity is related to deficiencies in fetal blood flow rather than abnormal trophoblastic cell function is suggested by the lack of difference in serum progesterone and hCG concentrations in women who abort compared with those who produce a live birth (Tables VII and VIII). Further support for the concept that trophoblastic dysfunction is an effect rather than a cause of abortion is gained from the observation that 89% of pregnancies associated with low progesterone concentrations displayed the absence of fetal heart activity at the time the low progesterone values were obtained.

Whatever the mechanism of the abortion, a single ultrasonographic examination at 6 weeks of gestation can be used to predict pregnancy outcome. Among pregnancies that abort, 58% have absent fetal heart activity at 6 weeks (empty sac or fetal pole without fetal heart activity) (Table V). Of the 42% of pregnancies

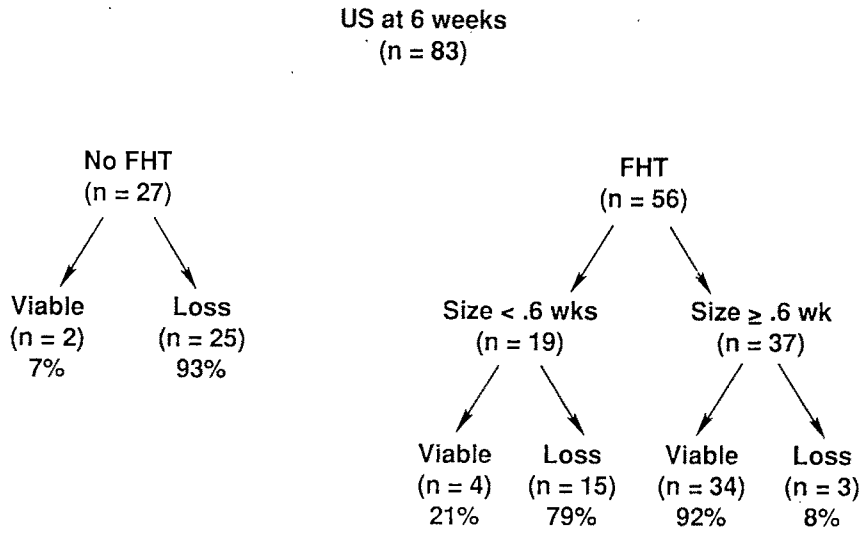


Fig. 1. Correlation between ultrasonographic findings at 6 weeks' gestation and subsequent pregnancy outcome. *FHT*, Fetal heart activity.

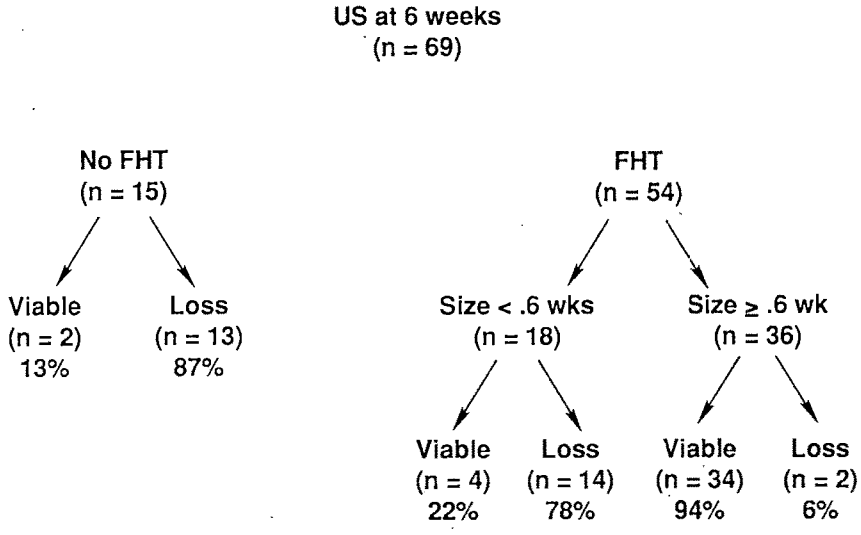


Fig. 2. Correlation between ultrasonographic findings at 6 weeks' gestation with subsequent pregnancy outcome among women with history of recurrent spontaneous abortion. *FHT*, Fetal heart activity.

Table X. Correlation between ultrasonographic findings at 6 weeks' gestation with pregnancy outcome in 14 pregnancies in which serum progesterone concentrations were <10 ng/ml at 6 weeks

	Viable pregnancy		*Abortion*			
	Recurrent spontaneous abortion		*Recurrent spontaneous abortion*		*No history of abortion*	
Ultrasonographic finding	*No.*	*%*	*No.*	*%*	*No.*	*%*
No fetal heart activity	0	0	5	83	3	100
Fetal heart activity	5	100	1	17	0	0
Fetal pole ≥0.6 wk	4	80	0	0	0	0
Fetal pole <0.6 wk	1	20	1	17	0	0

that abort after demonstration of fetal heart activity, 86% are <0.6 week than expected age on the basis of the LMP (Table VI). Only three of 43 pregnancies (8%) that aborted were not predicted at the time of the 6-week ultrasonographic examination. Thus, on the basis of the finding of presence or absence of fetal heart activity and the size of the fetal pole, 92% of spontaneous abortions can be predicted by evaluating a single ultrasonographic examination at 6 weeks' gestation.

Fig. 1 outlines how a single ultrasonographic examination at 6 weeks' gestation can be used clinically. Of 83 pregnancies examined 27 (33%) had an absence of fetal heart activity, and 25 (93%) of these pregnancies were lost. The presence of fetal heart activity in a fetal pole measuring <0.6 week from LMP occurred in 19 pregnancies. Fifteen of these pregnancies (79%) terminated in abortion. Of 37 pregnancies displaying the presence of fetal heart activity and fetal size not <0.6 week from LMP, three (8%) aborted. Thus in diagnosing impending abortion a single ultrasonographic examination at 6 weeks had a sensitivity of 85%, a specificity of 93%, a positive predictive value of 92%, and a negative predictive value of 87%. These values can also be calculated for women with a history of recurrent spontaneous abortion (Fig. 2). Of the 69 pregnancies occurring in women with recurrent spontaneous abortion, 29 ended in repeat abortion and 40 produced viable births. Ultrasonographic examination of these 69 pregnancies at 6 weeks' gestation revealed the absence of fetal heart activity in 15 (22%) and the presence of fetal heart activity in a fetal pole measuring <0.6 week from LMP in 18 (33%). Repeat abortion occurred in 13 of the 15 (87%) pregnancies with absent fetal heart activity and in 14 of the 18 pregnancies (78%) with fetal heart activity in fetal poles measuring <0.6 week from LMP. Thus in diagnosing a repeat abortion in women experiencing recurrent spontaneous abortion, a single ultrasonographic examination at 6 weeks' gestation had a sensitivity of 85%, a specificity of 93%, a positive predictive value of 94%, and a negative predictive value of 81%.

We conclude that ultrasonographic findings describing mechanisms of abortion are different when pregnancy losses experienced by women with a history of recurrent spontaneous abortion are compared with those with a history of no previous abortion. In addition, a single ultrasonographic examination at 6 weeks is predictive of spontaneous abortion in 92% of pregnancy losses. It is hoped that the information gained from these studies will lead to efficacious treatment and to better diagnosis of recurrent spontaneous abortion.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

DR. LEEBER COHEN, Chicago, Illinois. This article compares a small group of 14 previously nonaborting women who aborted with a larger group of 69 patients with a history of recurrent spontaneous abortion. It was designed to test the hypothesis that early embryogenesis as evidenced by vaginal ultrasonography will differ between these groups. This study is important because it could help identify women who are at higher risk of

recurrent abortion. Presumably evaluation for immune causes of recurrent abortion could then be evaluated and therapy initiated.

The authors have two major findings. The first is that fetal heart activity is present more frequently (55% vs 14%, $p < 0.02$) at 6 weeks' gestation in women with recurrent spontaneous abortion with another recurrent abortion than in a group of previously nonaborting women who abort. Second, they observe that fetuses with demonstrated heart tones that subsequently abort have significantly smaller crown-rump lengths than expected 86% of the time.

Because the authors are using two or more consecutive spontaneous abortions as a definition for recurrent spontaneous abortion, it might be useful to provide the risk rates for consecutive abortion after one, two, and three losses from the literature.

The Material and Methods section doesn't give information on age, reproductive history, or treatment for infertility or reproductive loss. The control group of previously nonaborting women with abortions is quite small. In addition it is not clear where these patients were recruited. To be able to extend any conclusions to the general population the control group should include a subgroup of nonaborters with successful pregnancies. To elaborate on this point, the data presented reveal that 29 of 69 (42%) of the recurrent spontaneous abortion patients had repeat abortions. We cannot calculate the abortion rate in the previously nonaborting group.

The authors need to clarify why in Table I seven of 14 previously nonaborting aborters had fetal heart activity at some time during the first trimester, but only two of 14 of the same group had fetal heart activity at 6 weeks in Table V. Did the other five fetuses have delayed appearance of the fetal heart activity and subsequently abort? Table II could be eliminated and discussed in the text. Table III could be eliminated and would make an interesting topic for another article with larger numbers. Table IV should be expanded to include a group of previously nonaborting women and correlate the ultrasonographic findings among subsequent aborted and live birth pregnancies. Table V lacks a column for viable births after no history of abortion. Table VI should be revised. Figs. 1 and 2 should be revised. The mean, median, and standard deviation measurements for normal pregnancies should be provided. The percentages of pregnancies aborting then could be graphically demonstrated. A similar figure for recurrent spontaneous abortion pregnancies could follow. The data in Tables VII through X should be placed in a second article. A brief statement as to the findings could be referenced.

Overall, the authors have made a potentially interesting observation. Specifically, they describe an ultrasonographic finding of a small-for-gestational age crown-rump measurement in a fetus with fetal heart activity at 6 weeks that subsequently aborted. The authors' findings suggest that this may be due to immune factors.

The findings that fetuses with small-for-gestational-age crown-rump lengths are at risk for spontaneous abortion are supported by a recent article by Nazari et al.[1] The authors might discuss the finding of Bromley et al.[2] that small-for-dates sac size is associated with spontaneous abortion. Similarly, they might discuss the report that abnormalities of fetal heart activity rates in the early fetus have been associated with abortion.[3]

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Dr. Tariq Siddiqi, Cincinnati, Ohio. The stated purpose of this investigation by Drs. Stern and Coulam was to "study the mechanisms of early pregnancy loss." Although an interesting and somewhat unique investigation, the conclusions drawn from this study are open to question. The investigators do not define whether this was a prospective or a retrospective study, i.e., how was the study population selected? Are these 83 consecutive patients of whom 69 happened to be recurrent aborters? If so, then a 100% abortion rate in patients with no history of abortion appears excessive. What were the inclusion or exclusion criteria? Were the patients who had live births relatively similar to those who aborted in regard to maternal characteristics such as age, gravidity, parity, and medical or surgical complications? If not, then we may be at risk of comparing apples and oranges.

The authors also do not define whether all patients were serially studied from 4 to 12 weeks of gestation, i.e., is this a longitudinal or a cross-sectional study? Ultrasonographic instruments, even transvaginal transducers, have inherent limitations such that a frequency of 7 MHz, the average lateral resolution is 1.3 mm. To this we may add the average standard deviation of 1.2 mm of three crown-rump length readings as stated by Robinson[1] in his original article analyzing the accuracy of crown-rump length measurements. Also, Robinson himself[2] was concerned about the reliability of measurements <12 mm, i.e., at <7 weeks gestation. The authors do not provide any information regarding their actual crown-rump length measurements nor do they make a distinction between observer and observation differences. This information is critical to evaluate their data, because a single measurement at 6 weeks' gestation will be in a range <10 mm, and on the basis of published data subject to a measurement error of ±2.5 mm. The data would be more meaningful when compared as the mean ± SD crown-rump length measurements in the live birth versus the abortion groups. It is therefore difficult to understand why the investiga-

tors chose to present their data as "weeks' gestation" rather than as actual crown-rump length measurements in millimeters. In fact, Robinson's ability to predict gestation is subject to a limitation of ±4.7 days, and 0.6 weeks falls within this error range. Finally, there are no statistical methods described in the manuscript.

As clinicians, we know LMPs are often inaccurate, yet these are used as a gold standard for estimating gestational age in this study, with ultrasonographic measurements being the dependent variable. How reliable are the LMPs in the study patients or are fertilization dates known to the investigators? In my opinion, the ultrasonographic observations are completely unrelated to the authors' hypothesis of fetal starvation as the mechanism underlying spontaneous abortion. They have neither performed biopsies of the implantation sites nor evaluated blood flow ultrasonographically to even speculate that blood flow is diminished in cases of spontaneous abortion. In fact, seven of the abortuses had an abnormal chromosone analysis, suggesting mechanisms other than diminished blood flow. Finally, are the authors really suggesting that in the event of a slightly smaller-than-expected crown-rump length measurement at 6 weeks' gestation and in light of known technical limitations spontaneous abortion is almost inevitable?

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Dr. Stern (Closing). This was a prospective unselected study. These patients were seen at the clinical office of the center from Jan. 1 to Dec. 31, 1990; we performed a retrospective analysis on the basis of pregnancy outcome. The mean age of the patients was 32 years, and the mean gravidity was 3.6. The pregnancies were followed starting on the fourth week of gestation until the end of the first trimester. All the pregnancies were studied with a transvaginal ultrasonography and blood test every week.

The results were reported by gestational age because this parameter is the most commonly use in clinical practice. The mean length of the normal fetal pole at 6 weeks was of 5 mm, with a range between 2 and 8 mm. Fisher's exact test was used for the statistical analysis.

The purpose of this study was to offer the clinician a tool to predict fetal outcome. We agree that knowing the exact time of conception will allow a more specific test. The problem is that in the clinical setting, the patient does not know the date of conception.

We grouped the patients with chromosomal abnormalities in one group trying to observe a difference in the ultrasonographic findings. We did not see a difference.

Our hypothesis is that recurrent spontaneous abortions have an immunologic component that activates the coagulation system, clotting the vasculature of the placenta; this will produce inadequacy in the growth of the fetus, giving a small fetus.

Acute childbirth morbidity: Its measurement using hospital charges

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OBJECTIVES: An analytic descriptive analysis of acute childbirth morbidity was carried out at Duke University Medical Center, comparing patients delivered by primary cesarean section with those delivered vaginally.

STUDY DESIGN: All primary cesarean deliveries and vaginal deliveries from July 1, 1981, through June 30, 1986, were combined with maternal and infant charge data. A total of 7256 patients were analyzed. A description of the charges for the associated diagnoses was carried out. A morbidity index was used to identify differences in predicted median hospital charges with 95% confidence intervals.

RESULTS: The ratio of mean primary cesarean delivery to mean vaginal delivery total charges was 2.5:1. The magnitude of the mean hospital charges was inversely related to the frequency of the indication with the lowest charges associated with dystocia and the highest with multiple pregnancy. Antepartum risk factors (increased maternal age, patient referral) were associated with increases in maternal and infant morbidity as measured by the morbidity index. Chronic maternal hypertension resulted in decreased maternal morbidity but increased infant morbidity when primary cesarean delivery was used. Although preterm delivery was associated with large increases in charges, it was not significantly altered by using primary cesarean delivery. Risk factors associated with the management of abnormalities of labor were associated with deceases in maternal and infant morbidity when primary cesarean delivery was used.

CONCLUSION: Analysis of acute childbirth morbidity, as measured by hospital charges, showed marked variation of diagnosis and risk-specific charges for patients delivered by primary cesarean section. (Am J Obstet Gynecol 1992;166:1853-62.)

Key words: Childbirth, morbidity, cesarean, measurement

In the conclusion of the book *Costs, Risks, and Benefits of Surgery*,[1] the authors' first recommendation is that "Appropriate studies of the effectiveness of surgical treatment should be carried out for selected conditions, particularly those where uncertainty leads to professional disagreement." In spite of this recommendation, limited information exists regarding the costs of cesarean delivery.[2-8] This study describes childbirth morbidity at Duke University Hospital by a retrospective comparative analysis of maternal and infant hospital charges for primary cesarean and vaginal deliveries. The rationale for this approach hinges on the use of appropriate statistical methods that can characterize this outcome measure.

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6/6/37125

Methods

A perinatal outcome evaluation measurement should be a reliable, objective index that can be ascertained in the perinatal period, which relates to future health and well-being.[9] Hospital charges would appear to be such an index of acute morbidity severity in that it is an objective measure that can be determined in the perinatal period. This study explores the use of hospital charge data in a 5-year analysis from July 1, 1981, to June 30, 1986, comparing vaginal delivery to primary cesarean delivery. As described in other clinical economic studies,[10] a comprehensive summary of costs or charges is not intended.

In a previously published paper,[11] the obstetric profile of patients cared for at Duke University Medical Center was described. Determinants of the prevalence of primary cesarean delivery were described as either major diagnostic categories or as risk factors. The hospital information system archives from July 1, 1981, to June 30, 1986, for both infants and mothers were searched for the hospital charge data and matched to the existing data set of clinical information with hospital identification codes for the mother and the date of delivery.

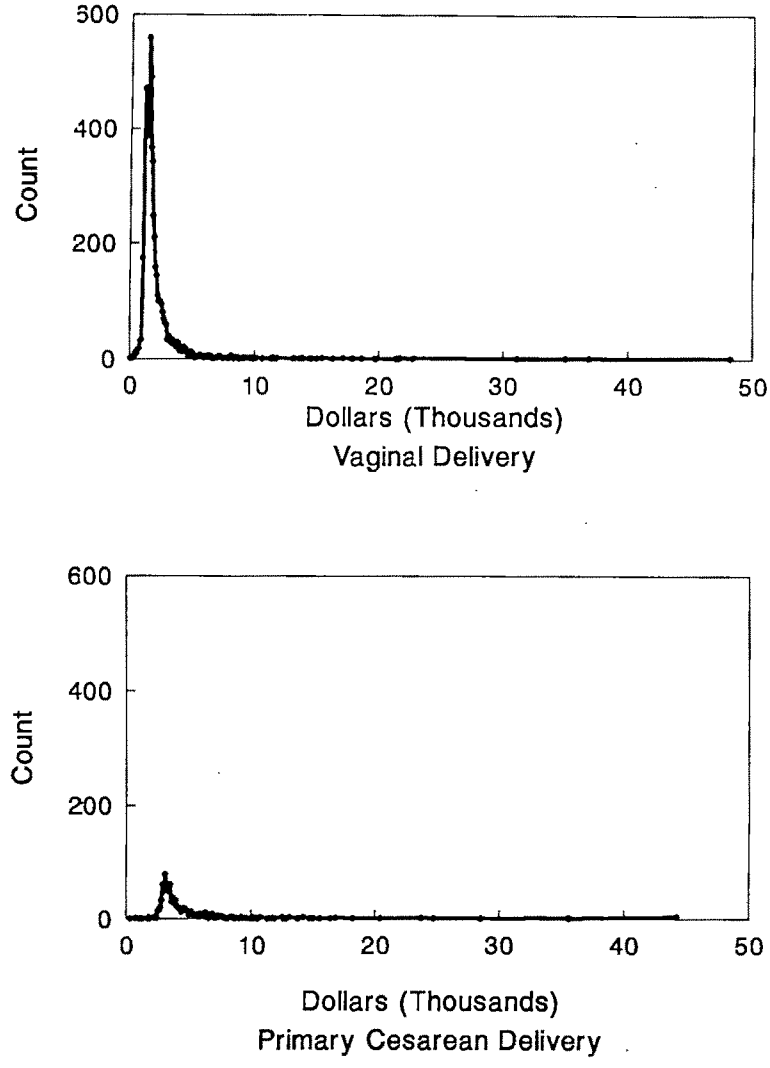


Fig. 1A. Distribution of maternal charges for primary cesarean and vaginal delivery at Duke University from 1981 through 1986.

Table I. Obstetric profile of infants delivered at Duke University Medical Center, July 1, 1981, through June 30, 1986

	*1981**	*1982*	*1983*	*1984*	*1985*	*1986†*	*Total studied*	*Total No.*
Primary cesarean delivery	87	160	236	244	265	159	1151	1339
Vaginal delivery	627	946	1261	1214	1356	701	6105	6808
TOTAL (%)								7256 (89.1)

*Last 6 months of 1981.
†First 6 months of 1986.

Because the hospital charge data did not meet normal distribution requirements, transformations of the data were explored in an attempt to satisfy normality requirements of the general linear models statistical procedures. These transformations proved difficult. A nonparametric method, analysis of the ranks of the charges, was next used to describe differences between groups. A distribution-based analysis was finally used, a proportional hazards model assuming a Weibull distribution of hospital charges. This seemed to fit the distribution of the hospital charges. Model testing was carried out in a random sample of the data and com-

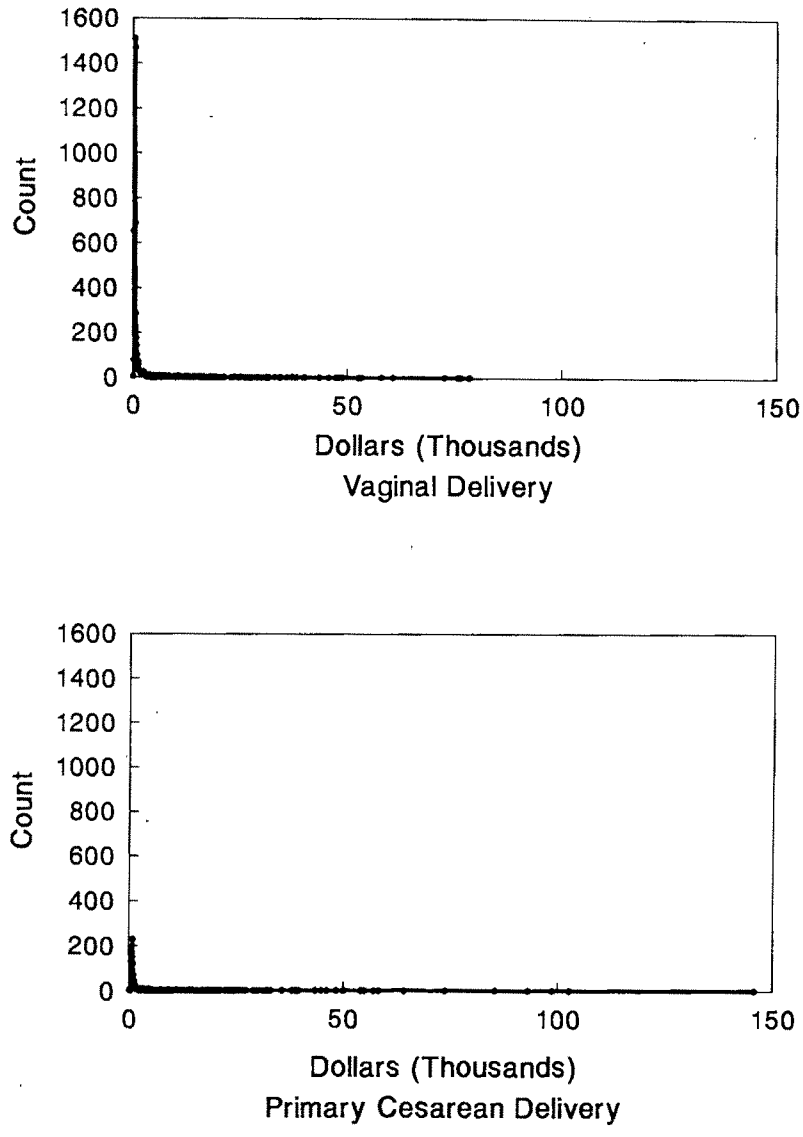


Fig. 1B. Distribution of infant charges for primary cesarean and vaginal delivery at Duke University from 1981 through 1986.

pared with the remaining data. Predicted median hospital charges with their 95% confidence intervals for selected antepartum and intrapartum risk factors were used to compare the effects of the method of delivery and the risk status for selected antepartum and intrapartum risk factors. The data were analyzed with the Statistical Analysis System release 5.18 and 6.03 (SAS Institute, Inc., Cary, N.C.).

Results

Table I describes the sample of primary cesarean and vaginal deliveries in the study sample by year. This sample captured 89.1% of vaginal and primary cesarean deliveries for the period of study. A higher ascertainment would have been more likely if an account code would have been present in both data sets. The distributions of maternal and infant charges are displayed in Figs. 1A and 1B. Distributions of charges by route of delivery for both maternal and infant charges are highly skewed to the right. Mean hospital charges for each year, type of delivery, and for both mother and infant are displayed in Fig. 2. Charges seemed stable for vaginal deliveries and for maternal charges for cesarean deliveries. Larger variations occurred in the infant charges for cesarean deliveries. No linear secular trends are observed. The ratio of mean primary cesarean to mean vaginal delivery charges was 2.5:1.

Table II. Predicted median hospital charges in dollars with the 95% confidence interval for selected antepartum and intrapartum risk factors at Duke University Medical Center fom 1981 through 1986

Risk factor status	*Vaginal delivery*		*Primary cesarean delivery*	
Maternal charges				
Referral				
Absent	1696 ± 39		3557 ± 169	
Present	2525 ± 161		5706 ± 508	
Nulliparity				
Absent	1789 ± 46	NS	4490 ± 268	
Present	1747 ± 59		3397 ± 214	
Maternal age ≥30 yr				
Absent	1707 ± 33		3602 ± 171	
Present	2244 ± 104		5454 ± 465	
Gestational age <37 wk				
Absent	1616 ± 33		3275 ± 152	
Present	3337 ± 156		6084 ± 425	
Chronic hypertension				
Absent	1723 ± 33		3931 ± 184	NS
Present	2441 ± 170		4305 ± 456	
Prolonged latent phase				
Absent	1770 ± 33	NS	4017 ± 180	NS
Present	1980 ± 225		3592 ± 547	
Late decelerations				
Absent	1787 ± 43	NS	4185 ± 204	
Present	1688 ± 114		3281 ± 293*	
Oxytocin induction				
Absent	1654 ± 33		3979 ± 194	NS
Present	2334 ± 104		4066 ± 351	
Infant charges				
Referral				
Absent	584 ± 23		1603 ± 132	
Present	901 ± 95		4685 ± 723	
Nulliparity				
Absent	644 ± 29	NS	2206 ± 238	
Present	710 ± 43		1688 ± 193*	
Maternal age ≥30 yr				
Absent	674 ± 28	NS	1955 ± 174	NS
Present	664 ± 54		2434 ± 394	
Gestational age <37 wk				
Absent	505 ± 15		998 ± 71	
Present	3739 ± 257		7594 ± 811	
Chronic hypertension				
Absent	664 ± 26		1784 ± 151	
Present	855 ± 107		3724 ± 732	
Prolonged latent phase				
Absent	668 ± 25		2121 ± 173	
Present	988 ± 203		1265 ± 349*	
Late decelerations				
Absent	675 ± 26	NS	2241 ± 200	
Present	683 ± 82		1440 ± 231*	
Oxytocin induction				
Absent	648 ± 26		2262 ± 202	
Present	811 ± 65		1411 ± 223*	

These predicted median charges were calculated with the SAS procedure Lifereg with the Weibull distribution for hospital charges. *NS*, Not significant.

*Significant reduction in charges within a given method of delivery.

Fig. 3 illustrates the distribution of mean charges by major diagnosis with the frequency of occurrence of the diagnostic categories for primary cesarean delivery. Total charges are inversely related to the frequency of occurrence for these diagnoses, with the highest charges for multiple pregnancy and the lowest charges associated with dystocia. Most of the variation was related to infant charges.

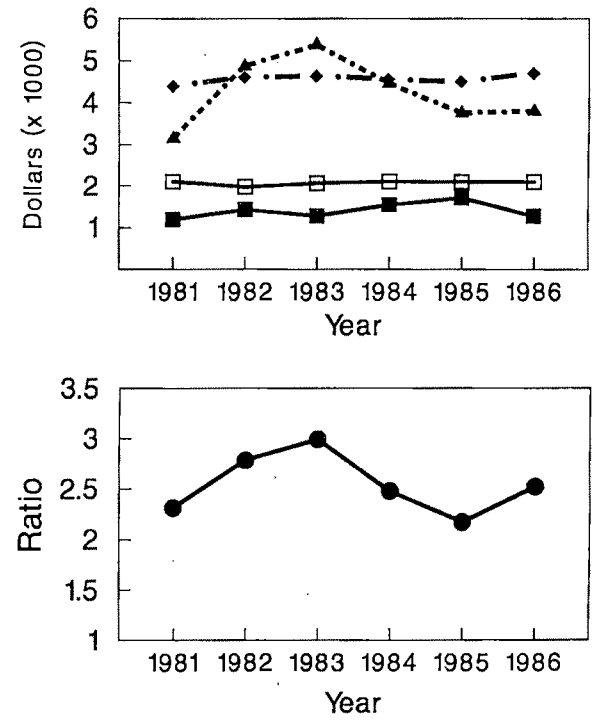


Fig. 2. Mean hospital charges and ratio of cesarean/vaginal charges. During study period, no overall trend is demonstrated for mean hospital charges for either infants or mothers. Variation in charges is specifically related to changes in charges of infants delivered by cesarean section.

Table II lists the predicated median hospital charges for the fitted model and their 95% confidence intervals for selected risk factors that had previously been identified as significantly affecting primary cesarean delivery rates. The effect of the risk factor and the method of delivery usually significantly increases charges, with more profound effects for infant charges. Notable exceptions, no significant difference, were observed for maternal vaginal delivery charges for nulliparous women, prolonged latent phase of labor, and late decelerations. Also, no significant differences were observed for maternal primary cesarean charges for chronic hypertension, prolonged latent phase of labor, and oxytocin induction. A significant reduction in predicted maternal charges was noted for primary cesarean delivery with nulliparous women and late decelerations.

Changes in predicted infant charges again showed significant increases with the risk factors, except for vaginal delivery infant charges for nulliparity and maternal age ≥30 years and late decelerations. Preterm delivery was associated with large increases in infant

charges, in spite of the method of delivery. Primary cesarean delivery infant morbidity was not significantly altered by a maternal age ≥30 years. Significant reductions in infant charges for primary cesarean delivery was noted for the risk factors of nulliparity, prolonged latent phase of labor, late decelerations, and oxytocin induction.

Fig. 4 summarizes the change in maternal morbidity for selected antepartum and intrapartum risk factors as a function of the method of delivery, i.e., primary cesarean versus vaginal delivery. The percent change was calculated as the ratio of the fitted predicted median charges of cesarean delivery to vaginal delivery with and without the risk factor. Maternal or infant indices that were close to unity were interpreted as showing no change in morbidity, those greater than unity as showing increased morbidity with primary cesarean delivery, and those less than unity as showing reduced morbidity with primary cesarean delivery. Fig. 5 summarizes the change in infant morbidity for the same factors as a function of the method of delivery.

The morbidity, as measured by hospital charges, shows both increases and decreases in morbidity with the use of primary cesarean delivery, with larger overall effects on infant morbidity. Mothers ≥30 years old and referral patients demonstrated increases in maternal and infant morbidity, whereas patients with intrapartum abnormalities (late decelerations, prolonged latent phase of labor, and oxytocin induction) were associated with decreases in maternal and fetal morbidity. Nulliparity was associated with decreases in maternal and infant morbidity. Preterm deliveries with primary cesarean delivery did not significantly change relative infant morbidity and only slightly reduced maternal morbidity. In contrast, primary cesarean delivery for patients with chronic hypertension significantly reduced maternal morbidity but increased infant morbidity.

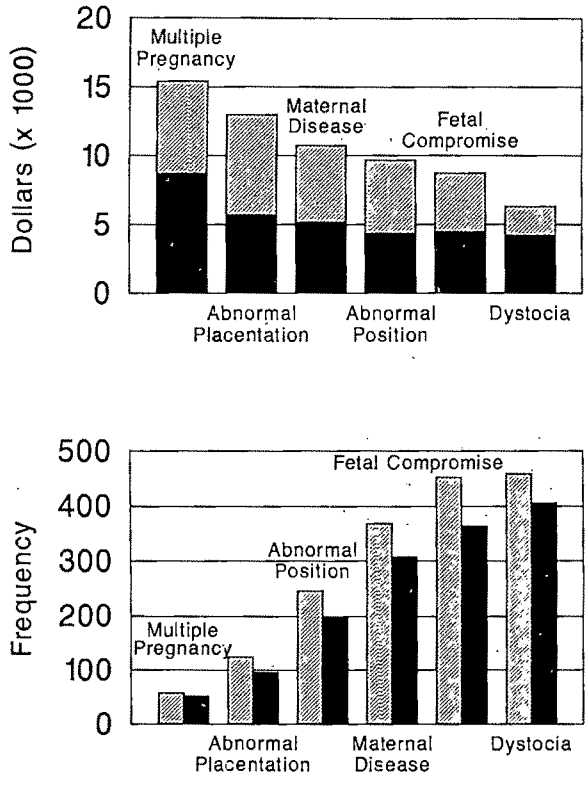


Fig. 3. Major diagnostic categories of primary cesarean delivery. Mean hospital charges and frequency of occurrence. The inverse relationship of total hospital charge to frequency of occurrence of major diagnostic category is demonstrated. *Solid bar,* Maternal; *hatched bar,* infant.

Comment

Two issues are explored in this study: (1) the use of hospital charges as a measure of acute childbirth morbidity, and (2) the hospital charges associated with primary cesarean and vaginal deliveries at Duke University over a 5-year period.

Hospital charges as a measure of acute childbirth morbidity. Childbirth morbidity can be defined as a continuum of outcomes that require the use of increased resources to restore the mother and infant to a normal state of health. It has been time limited to those events that affect the health of the mother or infant from the onset of labor to 6 weeks post partum. Consequently, the key to the measurement of morbidity is to find some measure that represents the use of resources around the time of childbirth.

The use of economic parameters to measure the effects of diagnostic variables on clinical outcomes has been infrequent. Charges have been seen as a measure of process and so poorly standardized they precluded comparisons within or between groups. In this study the temporal stability of hospital charges fulfilled an important criterion for any measure of outcome validity, relative secular stability.

The statistical analysis of any economic outcome is limited by the distribution of this measurement and the type of analysis performed. Three types of analyses are described in the literature,[9] cost-benefit, cost-effectiveness, and cost identification. Each has a slightly different purpose. Cost identification seems to apply to this study of childbirth outcomes in that it enumerates the charges in the provision of this service. The statistical methods have usually used mean costs or mean charges with data that, as shown in this paper, is highly skewed. The identification of a parametric model with the proportional hazards approach has not appeared in the literature. This approach offers the advantage of being able to model the effects.

A population-based approach seems poossible in that most of the population giving birth in the United States

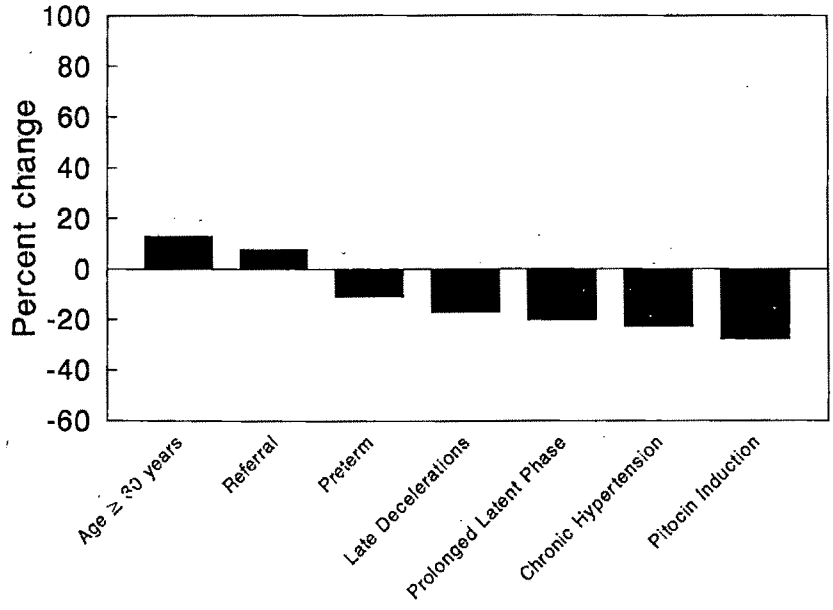


Fig. 4. Change in morbidity for mothers with use of primary cesarean delivery. Percent change was calculated from predicted median charges for both mothers and infants for each risk factor.

$$\text{Percent change} = \frac{\text{Predicted median (cesarean) with risk present/ Predicted median (cesarean) with risk absent}}{\text{Predicted median (vaginal) with risk present/ Predicted median (vaginal) with risk absent}} - 1 \times 100$$

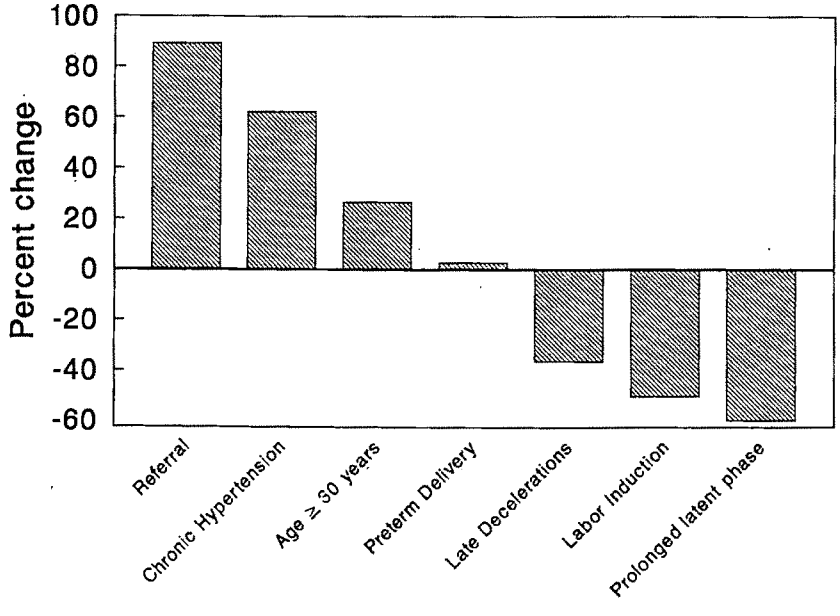


Fig. 5. Change in morbidity for infants with use of primary cesarean delivery.

does so within a hospital environment with known hospital charges. If used on a national level, standardization to national indexes could be used in comparisons between different geographic areas.

Childbirth morbidity of primary cesarean and vaginal delivery. The clinical question is the comparative question of the morbidity of cesarean versus vaginal delivery in risk-specific or disease-specific groups. Apgar scores, length of hospital stay, days on a ventilator, the occurrence of maternal fever, neonatal seizures,

and oxygen requirement are familiar categoric measures of outcome. Disease-specific but limited tools have been developed.[12] A more universal, continuous measure, applying both to the mother and infant, seems preferable over categoric measures or scores with arbitrary weighting of the variables. Cost identification would be such a method if it is useful in capturing the resources used and their relationship to future health status. This paper does not address the long-term implications of this charge identification but is the next logical step in evaluation of this measure. It should also be noted that hospital charges are only indicative of resources used and not of total perinatal cost.

Great controversies have developed around the methods of childbirth, usually centered on the questions of mortality and morbidity with little objective comparative data. A standardized, population-based parameter may help resolve the morbidity aspect of these difficult clinical questions given specific risk factor or diagnostic information. The clinical challenge is to choose the method with both the lowest mortality and the lowest short- and long-term morbidity given specific risk information for both the mother and infant.

This study demonstrated the utility of using hospital charges as a measure of acute childbirth morbidity during a period when the frequency of primary cesarean delivery was increasing. The major factor apparently responsible for this increase was an increasing concern about fetal outcome.[11] This analysis would support the use of primary cesarean section for the common intrapartum indications. Less clear would be the use of primary cesarean delivery for patients with chronic hypertension and in patients with a preterm infant. Antepartum risk factors affect the incidence of the use of the procedure and its associated morbidity.

Because it is unlikely that prospective, randomized studies will be carried out for primary cesarean delivery for a given diagnosis or risk factor, the strategy for evaluation of this technology will likely fall to comparative studies of outcome.

The necessity of a reliable measure of morbidity seems obvious. Population-based studies of diagnostic- or risk-specific delivery procedures should be carried out along with longitudinal studies of long-term outcome of this technology. The use of these methods could be applied to other hospital-based technologies.

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Discussion

Dr. Stephen S. Entman, Nashville, Tennessee. The issues that Dr. Hage raises may be the most important issues that will be discussed in this meeting and will progressively dominate all medical meetings. Although the current focus of attention for health care economists relates to the unfunded and underfunded segment of the population, it is very clear that there are only finite resources available for taking care of our population. Expenditures in one area will necessarily detract from the resources available for other areas. It is incumbent on us, as leaders within our specialty, to scrutinize the practice patterns that we have previously endorsed, many of which were initially well intended but have since been extrapolated beyond that original intent and applied without attention to either cost or real benefit. Thus Dr. Hage's quote from Dr. Bunker's book relating to the effectiveness of treatment, particularly where uncertainty leads to professional disagreement, is the critical point for the 1990s.

Although I agree with the spirit of Dr. Hage's topic, I have some semantic and methodologic concerns about the way in which he developed the theme in the manuscript. I have a semantic concern about the title and the attempt to relate charges as an index of morbidity. Morbidity is defined as relating to disease or to disease caused by an event. It would seem to me that Dr. Hage's use of the term morbidity primarily refers to the financial health of the individual and of society and not to an index of disease state. A title such as "Financial implications of clinical decisions: Cesarean section" might be more true to the purpose.

I also have several questions about the methodology used. The first relates to Dr. Hage's use of hospital charges rather than hospital costs. In my experience, hospitals have charge structures that do not necessarily reflect costs or even resources; there are markups that are differentially calculated for different activities. Specifically, vaginal deliveries are frequently charged with minimal markup, as a loss leader to draw families into the hospital care network. Frequently disposable items, more of which are used during a cesarean section, are marked up as a major profit center. Therefore, unless the ratio of charges to costs was consistent between the two modes of delivery, there may be bias in the data as presented.

My second question relates to the possibility of a systematic bias in the attrition of patient data from the sample. Although data were unavailable from 11% of all deliveries, 14% of cesarean sections were lost compared with only 10% of vaginal deliveries. This is both statistically and quantitatively significant.

It is not clear from the manuscript how many patients were included in each category of risk factor for cesarean section. Was the analysis controlled for all variables that might influence the impact of an individual patient on the financial data set? For example, would a 31-year-old nulliparous woman who was referred for chronic hypertension at 30 weeks and who had oxytocin induction with a prolonged latent phase and subsequently underwent cesarean section for fetal distress be counted in all eight categories of risk? If so, her data will affect the distribution of data in every category.

One of the risk factors noted was referral of patients. Some of these patients, as well as some indigenous patients, probably had antepartum hospitalizations up to the date of delivery. Were the charges for these antepartum stays removed from the data analysis? These charges, which could be considerable, are effectively independent of the decision for route of delivery. Therefore, it would be inappropriate to include them in an analysis of resources associated with cesarean section. Similarly, although fetal condition may influence the mode of delivery, neonatal charges are essentially independent of route of delivery. As was noted, the infant charges can have a significant impact on the total charges. Whatever point one might make about the excessive use of resources for cesarean section can be significantly blunted by the magnitude of antepartum and neonatal charges, both of which are governed more by severity of the underlying condition than by the delivery.

I certainly support Dr. Hage's attempts to address these issues. I would suggest that he apply corrections to the financial data to account for case mix severity and length of stay and to segregate the phases of care and address them independently. With these corrections, the data may normalize and allow him to express consumption of resources in units that may be more relevant to the question: mean charges per day and mean charges per discharge. I hope that he will pursue this effort to improve our understanding of how costly our obstetric practice patterns are and to encourage us to confirm the real benefits of the things we do to take care of patients.

Dr. Larry P. Griffin, Indianapolis, Indiana. This very complex examination of the relationship between hospital charge data and acute morbidity related to childbirth clearly demonstrates the difficulties with which health planners are faced in attempting to predict future health care costs.

In 1990 Richard D. Lamb, Director of the Center for Public Policy and Contemporary Issues at the University of Denver, published a discussion of a number of economic issues that face all of us in the practice of medicine as we enter the next century.[1] Nearly 12% of the gross national product in the United States is spent on health care, and health care costs continue to rise at a rate far greater than for other goods and services. In addition, age- and sex-adjusted mortality rates have not decreased in proportion to this dramatic increase in medical care costs. Perhaps as important is the fact that tax funds, either state or federal, provide 42% of the nation's health resource funds, a dramatic increase from the 26% provided in 1965. As a result, payors, both private and public, are closely examining the manner in which health care dollars are being spent.

Reproductive related health care constitutes 5.5% of the total health care expenditures in this country.[2] The largest categories of reproductive-related health care expenditures are obstetric care, which comprises 46% of reproductive health expenditures, and infant care, which is another 37% of these costs.

> To paraphrase Rashi Fein, we live in a society, not an economy. We must ultimately look at the larger canvas and decide; how do we spend limited resources to the highest benefit?[1]

We clearly are faced with a challenge.

Among other, more politically sellable and (to physicians) emotionally appealing health care solutions, such as reforming tort law, is the emphasis on more effective uses of health care dollars.

The American Medical Association is currently attempting to develop practice parameters in conjunction with many specialty societies to help to provide a yardstick for appropriate types of care in certain conditions. Although these exercises have some theoretic benefit in providing protocols for some standardized methods of care, many argue that this cookbook type of medicine tends to breed mediocrity rather than support the innovative approach that has served American medicine so well.

More apparent, however, are the economic benefits to be obtained by establishing practice parameters for specific conditions. The issue seems not whether we

need to evaluate the cost and therapeutic effectiveness of our approaches but rather how best to do this.[3,4]

Dr. Hage and his coinvestigators, along with others, have previously described significant changes in the rate of performance of cesarean delivery,[5] as others have done in regard to numerous other types of invasive procedures. Clearly, the conclusions of this article are useful. More important, however, is the demonstration of an approach to begin to analyze in some systematic, objective fashion the economic aspects of health care related to obstetrics and the multiple variables that must be considered in producing a useful model.

There are some limitations of the study, however. The failure to take into consideration over the period of the study the increases in charges related to inflation, incremental differences in the rate of change of specific hospital charges, changes in the length of stay for a given condition, and charges for outpatient services in addition to others bring the absolute reliability of the conclusions of the study into question. In addition, changes in the patterns of obstetric practice might be expected to produce different charges for the same diagnosis. Finally, prophylaxis for potential complications alone may be expensive, and no attempt was made to assess the cost of prophylactic measures on patients with specific risk factors, because many of these would be delivered in an ambulatory setting.

Additionally, the assessment of morbidity implies degree, yet no attempt was made to analyze indirect costs such as nursing care. For example, it costs an institution more in nursing time to take care of an ill patient than one who is simply occupying a bed, yet the basic charge for a hospital day is the same. Conversely, the most severe degree of morbidity, death, might be associated with lower hospital charges than seen in a patient with moderate complications.

As a result, I am concerned about the use of the term morbidity interchangeably with the concept of charges. This study in my opinion does not indicate they can reasonably be interchanged without significant confusion.

Finally, the huge differences between cities in charges for the same procedure, and even between different institutions within the same city, prevent this particular model from being a useful tool on a national or even regional basis without significant sophistication of technique. In an individual institutional setting, however, there may be useful extensions. Such an analysis, for example, might be used as an indicator of quality assurance to reflect various levels of high-risk care in response to the frequent justification for high cesarean delivery rates because of the high-risk nature of the patient population.

The authors are to be congratulated and encouraged in their work. As increased emphasis is placed on the cost effectiveness of various treatment modalities, and, as the development of practice parameters continues, accurate analysis of the economic costs of our treatment decisions is critical if we are to play a role in planning the future of health care delivery in this country.

I do have a couple of questions for the authors. (1) Have you attempted to use this technique on a total care basis (i.e., inpatient and outpatient for a specific procedure and diagnosis, visiting nurses, pharmaceuticals) to compensate for trends toward early hospital discharge and ambulatory or home care? (2) Might not costs be a better indicator than charges because charges vary much more from institution to institution than do costs for providing the service? (3) How can you reasonably adjust for interhospital differences in charges, which have been shown to range up to 1600% markups for some items in some institutions? (4) What do you see as the future direction of these types of economic studies in your institution?

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DR. HAGE (Closing). The issue of the economic aspects of care are going to continue to be an important part of the real world of practice, and how we respond is going to be very important.

Let me address what I think is a common issue of both the discussants, the semantics and the meaning of morbidity. It's difficult to have a clear definition of morbidity. A practical definition that I used was the increased use of resources to attempt to bring the patient back to a healthy state. That is a somewhat arbitrary definition and certainly still hypothetic. The reason for this definition is to attempt to encompass the costs of morbidity and its severity. As has been correctly pointed out, charges do not equal costs, so that hospital charges are only a proxy estimate of morbidity and morbidity severity.

Second was the question of bias because of the loss of patients and the method of analysis. The loss of information was related to the merging of two data sets and not from any exclusion of patients. I don't know of any way to prevent that except that as data bases are being developed multiple unique markers of patients and their babies should be included

Last, the use of hospital charges has beeen reported in the evaluation of the effectiveness of a perinatal intervention. Two examples are artifical surfactant

therapy[1] and the use of β-sympathomimetics for prevention of premature labor.[2] Their analysis methods have not been able to get around the problem of the skewed distributions of charges. The statistical method of this paper allowed the modeling of these distributions and the development of the morbidity index with the predicted median and its 95% confidence intervals. The utility of this method and this type of study should be verified at other centers and with other data sets of perinatal information.

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Editors' note: Readers may note we are publishing more Letters to the Editors in an effort to reduce the backlog and improve the publication time.

Treatment of fetal supraventricular tachycardia with flecainide acetate after digoxin failure

To the Editors: After reviewing the interesting case report by Kofinas et al. (Kofinas AD, Simon NV, Sagel H, Lyttle E, Smith N, King K. Treatment of fetal supraventricular tachycardia with flecainide acetate after digoxin failure. AM J OBSTET GYNECOL 1991;165:630-1), readers may be interested in a further case of fetal supraventricular tachycardia treated by maternal administration of flecainide after unsuccessful treatment with digoxin that was reported in the British literature.[1] A 26-year-old gravida 2, para 1 woman at 28 weeks' gestation had fetal tachycardia >200 beats/min on routine auscultation with a Pinard stethoscope. Fetal M-mode echocardiography showed a ventricular rate of 240 beats/min with a 1:1 atrioventricular relation. The heart was structurally normal, but moderate pericardial effusion and ascites were noted. The mother was given three oral doses of digoxin 500 μg every 8 hours and then 500 μg daily. Echocardiography showed a persistent supraventricular tachycardia in spite of trough maternal serum digoxin levels of 1.5 μg/L and amniotic fluid levels of 0.2 μg/L. Flecainide 110 mg (1.4 mg/kg) was then given intravenously under maternal echocardiography and fetal echocardiographic control. Fetal sinus rhythm at 120 beats/min supervened. Oral flecainide 100 mg every 8 hours was substituted for digoxin treatment. Trough maternal serum flecainide concentration was 656 μg/L (adult therapeutic range 400 to 1000 μg/L). The fetus stayed in sinus rhythm, and all traces of pericardial and ascitic effusion resolved within 10 days. Labor was induced at 38 weeks' gestation. A 12-lead electrocardiogram on the 3450 gm female infant showed sinus rhythm with no evidence of preexcitation. Concentrations of flecainide in maternal and cord plasma obtained simultaneously 5 hours after the last oral dose were 833 and 533 μg/L, respectively, confirming good transplacental passage of flecainide. The baby remained in sinus rhythm without further medication.

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REFERENCE

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Reply

To the Editors: I thank Mills for his interest in our article. We are aware of his case and another case published by Wren and Hunter.[1] Unfortunately, the JOURNAL allows only two references in brief communication articles, and we chose the two that were most appropriate for our paper.

We believe that flecainide acetate is a safe alternative for fetuses with hydropic changes unresponsive to digoxin. Flecainide acetate has been associated with lethal ventricular tachyarrhythmias in patients who received it to suppress arrhythmias caused by myocardial infarction. Certainly we must keep this in mind for maternal safety, but it is very unlikely that the heart of a healthy pregnant woman would be subject to the same effect. It is prudent, however, to administer this medication in a hospital setting under electrocardiographic observation of the maternal heart, especially when the medication is administered intravenously. Baseline electrocardiography before treatment with follow-up studies until therapeutic levels are achieved in the mother may be satisfactory in patients who receive the medication orally.

For these reasons it becomes clear that flecainide acetate should be used in the treatment of fetal supraventricular tachycardia only when other safer measures have failed to help the fetus. We hope that future cases will also be published so that we can gain more experience with the use of this medication and increase its safety margin.

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REFERENCE

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Amniotic fluid glucose and intraamniotic infection: Sensitivity, specificity, and predictive values

To the Editors: I read with interest the article by Kirshon et al. (Kirshon B, Rosenfeld B, Mari G, Belfort M. Amniotic fluid glucose and intraamniotic infection. AM J OBSTET GYNECOL 1991;164:818-20). It seems to me that in Table II the authors have incorrectly calculated the sensitivity, specificity, and positive and negative predictive value of their data. Nine patients had infections, and all had amniotic fluid glucose levels <10 mg/dl; this implies a sensitivity of 100% and not 75% as calculated by the authors. Likewise, 27 of 30 patients without infection were correctly identified by amniotic fluid glucose levels >10 mg/dl, a specificity of 90% and not 100%. Low glucose levels predicted 9 of 12 patients with infection, hence a positive predictive value of 75% and not 100%. Similarly, glucose levels >10 mg/dl correctly predicted the absence of infection in 27 of 27 patients, a negative predictive value of 100% and not 90%.

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Reply

To the Editors: We agree with Gonen's findings. Table II (page 819) should read as follows:

Positive predictive values	9/12 (75%)

Negative predictive values	27/27 (100%)
Sensitivity	9/9 (100%)
Specificity	27/30 (90%)

We thank Gonen again for his letter.

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Ureaplasma urealyticum resistance to erythromycin: Confirmed by clinical trial

To the Editors: The article by Eschenbach et al. (Eschenbach DA, Nugent RP, Rao AV, et al. A randomized placebo-controlled trial of erythromycin for the treatment of *Ureaplasma urealyticum* to prevent premature delivery. AM J OBSTET GYNECOL 1991;164:734-42) was inappropriately titled. It should have been titled "*Ureaplasma urealyticum* resistance to erythromycin: Confirmed by clinical trial."

Ostensibly a megabucks clinical trial was done involving five medical centers with the object of eradicating ureaplasmas in test patients versus placebo-treated patients. The reference to eradication by tetracycline was cited by the authors,[1] yet erythromycin was used in the study.

Strange that not one of the 29 people listed as either coauthors or members of the Vaginal Infections and Prematurity Study Group suggested the use of antibiotic susceptibility testing of the ureaplasmas before initiating antibiotic administration. It seems to be poor medical practice to treat empirically, especially because resistance to erythromycin is common among the current isolates of ureaplasmas in the United States.

Our laboratory isolates and processes approximately 5000 cultures of ureaplasmas and mycoplasmas a year and does antibiotic susceptibility testing routinely: we have found erythromycin resistance to be so high among *Ureaplasma* isolates that erythromycin is never suggested as appropriate therapy. In our latest 1600 consecutive antibiotic susceptibility tests of *Ureaplasma* isolates, 86% of the strains were resistant to erythromycin. Of 400 *Mycoplasma hominis* strains, 100% were resistant.

We are extremely pleased to see that our in vitro findings have been confirmed by in vivo trials.

Tetracycline and doxycycline are the antibiotics of choice assuming the microorganisms are susceptible (15% are not). Those antibiotics are not usually used during pregnancy. Ideally, therefore, the ureaplasmas and mycoplasmas should be eradicated before conception.

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REFERENCE

1. Elder HA, Santamarina BAG, Smith S, et al. The natural history of asymptomatic bacteriuria during pregnancy; the effect of tetracycline on the clinical course and the outcome of pregnancy. AM J OBSTET GYNECOL 1971;111:441-62.

Reply

To the Editors: Since the discovery of *Ureaplasma urealyticum* in 1937, reports on these microorganisms have generated more controversy than definitive results. *U. urealyticum* are ubiquitous in the female genital tract, and their isolation from 70% to 80% of pregnant women could even raise the question of whether they should be considered part of normal vaginal flora. They are commonly isolated from the chorioamnion of women with chorioamnionitis. In certain settings such as septicemia, pneumonia, and chronic lung disease in premature neonates, postpartum bacteremia in women and nongonococcal urethritis in men, *U. urealyticum* has been associated with disease. However, given their frequency in the vagina, the attack rate for disease appears to be exceptionally small.

We recently reported the results of a large National Institutes of Health–supported multicenter study of vaginal infections and prematurity in which *U. urealyticum* (in the vagina of 72% of >8000 women) was associated with no increase in prematurity or postpartum or neonatal disease.[1] It was not unexpected to find that the administration of erythromycin compared with placebo for a mean of 36 days also had no effect upon the incidence of prematurity or postpartum or neonatal disease.[2]

Kundsin et al. asked whether another antibiotic might have produced different results. Data from their laboratory suggest a high frequency of *U. urealyticum* resistance to erythromycin. Erythromycin was chosen to treat *U. urealyticum* in the National Institutes of Health study because it had previously been associated with a reduction of prematurity when used to treat *U. urealyticum*.[3] In contrast to Kundsin's data, others have consistently reported that erythromycin is active in vitro against *U. urealyticum*.[4,5] Further, erythromycin is one of the few drugs active against the microorganism that can be safely used in pregnancy. *U. urealyticum* is resistant to β-lactams because they lack peptidoglycan and to sulfonamides because they do not synthesize folic acid. As might be expected, differences of opinion occur over variations in susceptibility testing, but in most reports 72% to 97% of *U. urealyticum* are considered susceptible or moderately susceptible to erythromycin.[4-6] In comparison, moderate to good susceptibility has been reported to be 81% to 93% for tetracyclines, 97% for chloramphenicol, 47% for clindamycin, and 36% for ciprofloxacin.[4-6] Thus except for erythromycin, drugs effective in vitro are either contraindicated or too toxic for use in pregnancy.

If *U. urealyticum* is inhibited in vitro by erythromycin, why did prolonged erythromycin treatment not eliminate *U. urealyticum* from the vagina? Although we ad-

vanced numerous theories,[2] careful follow-up has revealed similar high-failure rates among pregnant women treated with erythromycin[3] and among nonpregnant women treated with tetracyclines.[5] Should we wait for more data? In clinical practice it would be difficult to recommend screening or treating vaginal *U. urealyticum* given the current data. Of course, if additional data become available this recommendation would be reevaluated.

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Umbilical artery flow velocity after vibratory acoustic stimulation

To the Editors: We read the article by Gagnon et al. (Gagnon R, Morrow R, Ritchie K, Hunse C, Patrick J. Umbilical and uterine artery blood flow velocities after vibratory acoustic stimulation. AM J OBSTET GYNECOL 1988;159:574-8) with considerable interest. They concluded that the decrease in umbilical peak-systolic/end-diastolic ratio was the result of fetal tachycardia rather than a change in the placental vascular resistance. We agree that umbilical artery blood flow velocity decreases significantly after vibratory acoustic stimulation; this decrease coincides with the significant increase in instantaneous fetal heart rate (FHR) value.

Our recent study (unpublished data, 1991), however, showed that there was significant decrease in resistance index in the umbilical artery even when resistance index was corrected with FHR value. In our series of 476 normal pregnant women there was a significant ($p < 0.001$) difference in mean resistance index in the umbilical artery before vibroacoustic stimulation and after stimulation. We corrected the resistance indexes of both groups according to FHR value with two methods.

We selected the samples of resistance index according to the same FHR value (150 to 160 beats/min) from both groups. The mean resistance index of prestimulation samples was 0.570 ± 0.071 ($n = 81$); that of poststimulation samples was 0.548 ± 0.067 ($n = 96$). The significant ($p < 0.001$) difference remained between two samples. The mean resistance indexes of two samples, however, might not reflect those of both original groups because these samples of both groups consisted of different cases.

Therefore we corrected the mean resistance indexes of the total cases ($n = 476$) by means of negative correlation between resistance index and FHR value. The mean corrected resistance index of prestimulation was 0.568 ± 0.066; that of poststimulation was 0.547 ± 0.063. The significant ($p < 0.001$) difference still remained between those two groups after correction.

These data suggested that any causes other than fetal acceleration might decrease the resistance index after vibroacoustic stimulation.

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Reply

To the Editors: We thank Nakamura et al. for their interest in our article and the opportunity to discuss some aspects of the relationship between instantaneous FHR value and umbilical artery Doppler flow velocity waveforms.

Our initial study was designed only to determine whether vibratory acoustic stimulation could significantly alter umbilical artery Doppler flow velocity waveforms. We concluded that vibratory acoustic stimulation decreases the umbilical artery peak-systolic/end-diastolic ratio at the same time FHR increases; our data suggested that the changes seen in umbilical artery peak-systolic/end-diastolic ratio after vibratory acoustic stimulation were caused by changes in FHR rather than changes in placental vascular resistance. Subsequently, similar observations have been made by Wladimiroff and Cheung[1] in the fetal internal carotid artery with the pulsatility index. However, when the pulsatility index was standardized for a FHR of 140 beats/min, the fetal internal carotid artery pulsatility index remained virtually unaltered. They concluded that vibratory acoustic stimulation did not alter fetal cerebral vascular resistance, but only FHR.

In a subsequent article we demonstrated that the relationship between FHR and umbilical artery peak-systolic/end-diastolic ratio was curvilinear and that FHR had a significant effect only on the end-diastolic component of the waveform and not on the peak-systolic component. The results displayed in Nakamura et al. regarding the effect of vibratory acoustic stimulation

on the umbilical artery resistance index, after correction for FHR being made, involved a large number of patients and demonstrated a modest effect (3.5% decrease) of vibratory acoustic stimulation on umbilical artery resistance index. Our previous study involved only 11 women; with a coefficient of variation between 6% and 8% during control observations, we would not have been able to detect such a small difference. In addition, with a large number of subjects it is well known that a small effect could be statistically significant but of no clinical or physiologic significance. The reverse is also true. With a small number of subjects, a large effect could be statistically insignificant but of high clinical or physiologic significance.

For these reasons we maintain our initial conclusion that vibratory acoustic stimulation does not alter placental vascular resistance; it only increases FHR with a subsequent increase in the end-diastolic component of the waveform. As a result of these changes in FHR any Doppler-derived index of vascular resistance of any fetal blood vessel would probably suggest a decrease in downstream vascular resistance after vibratory acoustic stimulation. However, interpretation should take into account other physiologic variables that could alter these waveforms, such as FHR.

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Steroids receptors in the endometrium during clomiphene citrate treatment

To the Editors: In a recent article Fritz et al. (Fritz MA, Holmes RT, Keenan EJ. Effects of clomiphene citrate treatment on endometrial estrogen and progesterone receptor induction in women. Am J Obstet Gynecol 1991;165:177-85) indicated that there has been no effect on endometrial estrogen and progesterone receptors in luteal phase after ovulation induction with clomiphene citrate 150 mg/day from cycle days 5 to 9 compared with normal ovulatory cycles.

We have awaited this logical observation since Aksel et al.[1] and Roennberg et al.[2] reported reduced levels of receptors after treatment with clomiphene citrate.

We have immunohistochemically examined the effect of adjuvant breast cancer treatment with another antiestrogenic agent, tamoxifen citrate, 40 mg/day on the postmenopausal endometrium and found that it markedly induced more progesterone and estrogen receptors when compared with an untreated control group (unpublished data).

This increase in progesterone and estrogen receptors may be caused by the partial estrogenic agonist action of these antiestrogens and may explain the increasing risk of endometrial carcinoma in this group of patients.[3]

The study by Fritz et al. also showed that both estrogen and progesterone receptors in the late luteal phase were similarly reduced in both normal ovulatory and clomiphene citrate–stimulated cycles. Press et al.[4] attributed this phenomenon to the suppressive effect on receptors associated with the increased level and activity of progesterone in the second half of the cycle.

Those who noted reduced receptors after clomiphene citrate in the infertile patient might have missed the improvement of progesterone secretion associated with the recruitment of more follicles in treatment cycles.

That the Fritz et al. study found no change in the receptor status in spite of the increased level of progesterone in the clomiphene citrate–stimulated cycle may represent a relative increase of receptors because the expected reduction of receptors did not occur. This finding is supported by our own observations.

Alternatively, the findings of Fritz et al. could be explained by the short duration of clomiphene treatment, hence the less vigorous progesterone level and activity (one cycle only). The duration of treatment and the clomiphene citrate dose might have an impact on the receptor status of the endometrium.[4]

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Reply

To the Editors: We thank Shabib and Pitrof for their interest in our work. Their immunohistochemical evidence for increased concentrations of endometrial estrogen and progesterone receptor in postmenopausal women receiving adjuvant breast cancer treatment with tamoxifen is interesting and consistent with our previous observations that estrogen receptor concentrations (as determined by quantitative immunoassay) are higher in breast cancer recurrences obtained from patients receiving tamoxifen.[1] Indeed, increased steroid receptor concentrations in these patients receiving chronic antiestrogen therapy might be attributed to the partial estrogen agonistic activity ascribed to tamoxifen or its metabolites. Alternatively, chronic antiestrogen

therapy may produce increased serum levels of estrogenic steroids in some breast cancer patients.[2]

There are several possible explanations for our findings of similar midcycle and late secretory phase endometrial estrogen and progesterone receptor concentrations in control and clomiphene treatment cycles. The suppressive effects of increased circulating progesterone levels or persistent estrogen antagonism by residual clomiphene may have prevented any increase in receptor induction that otherwise might accompany the markedly higher estrogen concentrations after clomiphene treatment. Conversely, maximal concentrations of endometrial steroid receptor may be induced by normal ovulatory threshold levels of estradiol; elevated estrogen levels may have offset any decline in receptors caused by the increase in progesterone or clomiphene's antiestrogenic action. As Shabib and Pitroff suggest, the absence of a reduction in receptor levels after clomiphene treatment in spite of the marked increase in serum progesterone could also, in part, reflect the estrogenic activity linked to clomiphene or a metabolite with similar activity.

Our data, together with those of Hecht et al.,[3] demonstrated that the many and competing direct and indirect effects of clomiphene treatment have no net impact on midcycle, midluteal, or late luteal endometrial steroid receptor concentrations in normally cycling women. We agree that dose- and time-dependent adverse effects of clomiphene treatment on endometrial steroid receptor populations have not been excluded. Although Hecht et al.[3] observed no adverse effects with either low- or high-dose clomiphene, our study clearly demonstrated the potential for significant systemic accumulation of clomiphene or its metabolites after repeated cycles of administration. Consequently the effects of experimental acute clomiphene administration in normal women may well differ from the effects in anovulatory individuals who receive longer-term treatment.

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The tip of the iceberg and the best fit in ultrasonographic dating of pregnancy

To the Editors: Goldstein (Goldstein SR. Embryonic ultrasonographic measurements: crown-rump length revisited. Am J Obstet Gynecol 1991;165:497-501) has revisited crown-rump length measurements and found that currently used tables for dating pregnancy are invalid at gestational ages of <8.5 weeks.

Whenever a new technical device is offered to the medical profession new methods are developed to make the device a usable tool. In the early stages the necessary time is not always taken to assure that these methods satisfy the most stringent criteria. Therefore these methods should be considered preliminary until better and more biologically correct methods are developed.

Admittedly, with the concomitant introduction of computers and obstetric ultrasonography, the fastest and easiest way to make dating equations was to take the measurements from the ultrasonogram and the supposedly good dates to a computer and try to obtain the best fit by using different polynomials in linear regression analyses. The best-fit equation became the dating equation used to make the tables.

Whether the parameter was crown-rump length, biparietal diameter, head circumference, or femur, there was a serious problem. Because polynomials cannot be extrapolated it became important to include as many early measurements as possible to expand the usefulness of the new dating equation. By measuring only the largest fetuses of the early population and not realizing that only the tip of the iceberg was measured investigators were blinded by very low random errors. The equations were generally accepted because the computers displayed excellent fits above anything seen in obstetrics before. Thus the "cult of the best fit" was born.

Methods are, however, not evaluated by their random errors. The systematic errors created by the selection of only the largest measurements in early pregnancy had to be balanced by an opposite systematic error later in pregnancy by the analytic method.

With an abdominal transducer and patient material with known date of ovulation examined and analyzed longitudinally, biologic (linear) dating curves were made for the gestational sac, crown-rump length,[1] biparietal diameter, mean head diameter,[2] and femur length.[3] Made before the general introduction of vaginal probes, these simple dating functions have proved to have full validity for measurements even before a gestational age of 6 weeks. Because of the rapid growth of the crown-rump length, the almost C-shaped curve of the fetus will not reduce the assessment of gestational age by >1 or 2 days, well within the variability limits.

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Reply

To the Editors: Rossavik's work in statistical analysis relative to pregnancy dating is well-known and respected, as is evidenced by his reference list. I am compelled, however, to make the following statements:

(1) The opinion I expressed was that use of the terminology "crown-rump length" is inappropriate before long-axis measurement of 18 to 22 mm when a true crown and rump are discernable.[1]

(2) The appreciation of the correct anatomic structures and appearance can only enhance the accuracy and scientific appropriateness of current or future measurements, especially in light of newer highly magnified endovaginal probes and the "sonomicroscopy" they afford.

(3) Rossavik states that "because of rapid growth of the crown-rump length, the almost C-shaped curve of the fetus will not reduce the assessment of gestational age by >1 or 2 days, well within the variability limits." When compared with Robinson and Fleming[2] our data suggest an *increase* in gestational age by 2 to 3 days[3] (presumably caused by the new understanding, and thus careful noninclusion, of the adjacent yolk sac). Although this is within variability limits, the effect is to shift the mean (and thus the entire confidence limit).

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Hydroxyurea and pregnancy

To the Editors: Patel et al. (Patel M, Dukes IAF, Hull JC. Use of hydroxyurea in chronic myeloid leukemia during pregnancy: a case report. Am J Obstet Gynecol 1991;165:565-6) reported a patient with chronic myeloid leukemia in whom hydroxyurea was used during pregnancy with a successful outcome for both mother and fetus. We would like to report a similar observation of a woman with chronic myeloid leukemia who was administered hydroxyurea at conception and throughout pregnancy.

Hyperleukocytosis was found during the course of a biologic assessment in a 30-year-old woman who complained of sterility. Chronic myeloid leukemia was diagnosed in August 1984. Results of the Philadelphia chromosome test were positive. None of her siblings were histocompatible and hydroxyurea was given (1 to 2 gm/day) while the search for an unrelated bone marrow donor was initiated. Four years later the patient became pregnant. After receiving precise information about the potential fetal risk of hydroxyurea treatment and about the prognosis of her disease, she eagerly refused a therapeutic abortion. During pregnancy hydroxyurea could not be discontinued in view of dose-dependent thrombocytosis and hyperleukocytosis; the dosage ranged from 1 to 3 gm/day. At 15 weeks of pregnancy cytogenetic analysis of amniotic fluid showed a normal 46,XY karyotype. Shortly after, splenalgia and increasing splenomegaly led to low-dose radiotherapy of the spleen (1.5 Gy), which had an obvious, albeit transient, beneficial effect. A cesarean section was planned at 38 weeks of gestation for obstetric reasons, and hydroxyurea was discontinued 1 week before delivery to alleviate a potential cytopenia in the fetus. The patient was delivered of a male infant with a birth weight of 3100 gm and normal clinical status who thereafter experienced normal growth and development (follow-up 32 months). Hematologic assessments of umbilical cord blood samples and blood samples taken during the first week of life showed normal results.

In our patient, as in the patient of Patel et al., continuous administration of hydroxyurea before and throughout pregnancy had neither teratogenic effects nor hematologic consequences on the fetus. Teratogenic effects of hydroxyurea have been demonstrated in mammals when given at the onset of gestation and at doses at least five times those commonly used in humans.[1-3] However, clinical reports in humans have shown that the risk of teratogenicity was often overestimated: In none of the hitherto published cases was any untoward effect observed on the course of pregnancy or on the fetus.[4]

Recent demonstration of the potential beneficial effects of hydroxyurea in sickle cell disease by the bias of a decrease in hemoglobin S/hemoglobin F ratio[5] has recently led to a reappraisal of the adverse effects of its chronic administration in young women. If available, other cases of hydroxyurea administration throughout pregnancy for hematologic malignancies should be collected before any consensus is made on new therapeutic approaches in young women with sickle-cell disease.

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Certification for these individuals is time-limited, and the period of certification is December 13, 1991, through December 31, 2001.

Cumulative total certificates awarded for this subspecialty: 30,957

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The names of 14 newly classified diplomats are not included in this listing at their request.

New certifications: Subspecialty of Gynecologic Oncology

Date of examination: December 1991

Place: Chicago, Illinois

Number certified this examination: 22

Cumulative total of certificates awarded for this subspecialty: 422

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New certifications: Subspecialty of Maternal-Fetal Medicine

Date of examination: December 1991

Place: Chicago, Illinois

Number certified this examination: 71

Cumulative total of certificates awarded for this subspecialty: 689

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New certifications: Subspecialty of Reproductive Endocrinology

Date of examination: December 1991

Place: Chicago, Illinois

Number certified this examination: 41*

Cumulative total of certificates awarded for this subspecialty: 448

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**The name of one newly classified diplomate is not included in this listing at his/her request.*

New certification: Subspecialty of Critical Care

Date of examination: December 1991

Place: Chicago, Illinois

Number certified this examination: 1

Cumulative total of certificates awarded for this subspecialty: 1

Van Hook, James W., Department of Obstetrics and Gynecology, Univ. Washington School Med., 1959 Pacific Ave., Seattle, WA 98195

Recertifications: Principal Specialty

Recertifications effective as of December 13, 1991

Cumulative number recertified: 4,698*

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November 16-21, 1992, 15th Annual OB/GYN Review, Costa Mesa, California
January 13-17, 1993, 8th Annual Perinatal Symposium, Beaver Creek, Oregon
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Vasodilation
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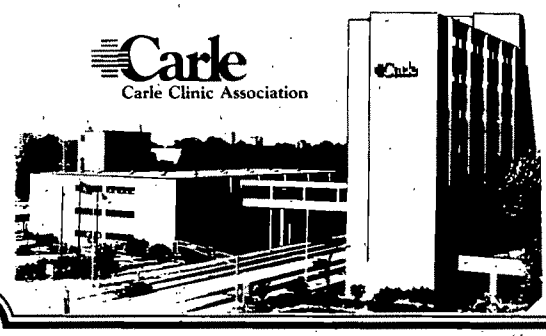
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Send inquiries and curriculum vitae to:

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Professor and Chairman
Department of Obstetrics and Gynecology
UMDNJ-Robert Wood Johnson Medical School
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J. A. Spinnato, M.D.
Director, Division of Maternal-Fetal Medicine
Dept. of OB/GYN,
University of Louisville
Louisville, KY 40292

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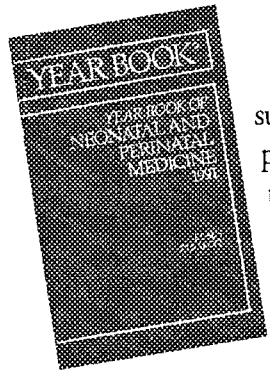
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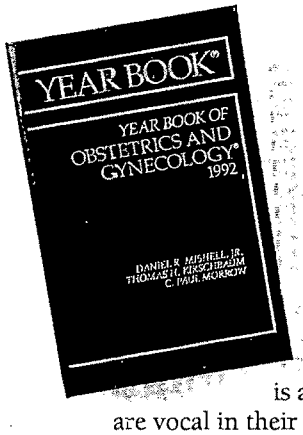
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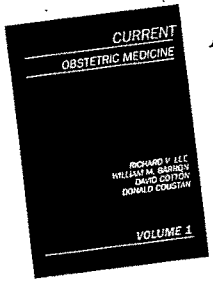
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INDICATIONS AND USAGE: PREVENTION OF PREGNANCY.

CONTRAINDICATIONS: Oral contraceptives should not be used in women who currently have the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophlebitis or thromboembolic disorders. 3. Cerebral vascular or coronary artery disease. 4. Known or suspected carcinoma of the breast. 5. Carcinoma of the endometrium or other known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Cholestatic jaundice of pregnancy or jaundice with prior pill use. 8. Hepatic adenomas or carcinomas. 9. Known or suspected pregnancy.

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risks of several serious conditions including myocardial infarction, thromboembolism, stroke, hepatic neoplasia, and gallbladder disease, although the risk of serious morbidity or mortality is very small in healthy women without underlying risk factors. The risk of morbidity and mortality increases significantly in the presence of other underlying risk factors such as hypertension, hyperlipidemias, obesity and diabetes. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks. The information contained in this brief summary is principally based on studies carried out in patients who used oral contraceptives with higher formulations of estrogens and progestogens than those in common use today. The effect of long term use of the oral contraceptives with lower formulations of both estrogens and progestogens remains to be determined. Throughout this brief summary epidemiological studies reported are of two types: retrospective or case control studies and prospective or cohort studies. Case control studies provide a measure of the relative risk of a disease, namely a *ratio* of the incidence of a disease among oral contraceptive users to that among nonusers. The relative risk does not provide information on the actual clinical occurrence of a disease. Cohort studies provide a measure of attributable risk, which is the *difference* in the incidence of disease between oral contraceptive users and nonusers. The attributable risk does provide information about the actual occurrence of a disease in the population. For further information, the reader is referred to a text on epidemiological methods. 1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS. a. Myocardial Infarction. An increased risk of myocardial infarction has been associated with oral contraceptive use. This risk is primarily in smokers or women with other underlying risk factors for coronary artery disease such as hypertension, hypercholesterolemia, morbid obesity, and diabetes. The relative risk of heart attack for current oral contraceptive users has been estimated to be two to six. The risk is very low under the age of 30. Smoking in combination with oral contraceptive use has been shown to contribute substantially to the incidence of myocardial infarctions in women in their mid-thirties or older with smoking accounting for the majority of excess cases. Mortality rates associated with circulatory disease have been shown to increase substantially in smokers, especially in those 35 years of age and older among women who use oral contraceptives. Oral contraceptives may compound the effects of well-known risk factors, such as hypertension, diabetes, hyperlipidemias, age and obesity. In particular, some progestogens are known to decrease HDL cholesterol and cause glucose intolerance, while estrogens may create a state of hyperinsulinism. Oral contraceptives have been shown to increase blood pressure among users (see section 9 in WARNINGS). Similar effects on risk factors have been associated with an increased risk of heart disease. Oral contraceptives must be used with caution in women with cardiovascular disease risk factors. b. Thromboembolism. An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Case control studies have found the relative risk of users compared to non-users to be 3 for the first episode of superficial venous thrombosis, 4 to 11 for deep vein thrombosis or pulmonary embolism, and 1.5 to 6 for women with predisposing conditions for venous thromboembolic disease. Cohort studies have shown the relative risk to be somewhat lower, about 3 for new cases and about 4.5 for new cases requiring hospitalization. The risk of thromboembolic disease associated with oral contraceptives is not related to length of use and disappears after pill use is stopped. A two- to four-fold increase in relative risk of post-operative thromboembolic complications has been reported with the use of oral contraceptives. The relative risk of venous thrombosis in women who have predisposing conditions is twice that of women without such medical conditions. If feasible, oral contraceptives should be discontinued at least four weeks prior to and for two weeks after elective surgery of a type associated with an increase in risk of thromboembolism and during and following prolonged immobilization. Since the immediate postpartum period is also associated with an increased risk of thromboembolism, oral contraceptives should be started no earlier than four weeks after delivery in women who elect not to breast feed. c. Cerebrovascular diseases. Oral contraceptives have been shown to increase both the relative and attributable risks of cerebrovascular events (thrombotic and hemorrhagic strokes), although, in general, the risk is greatest among older (>35 years), hypertensive women who also smoke. Hypertension was found to be a risk factor for both users and non-users, for both types of strokes, and smoking interacted to increase the risk of stroke. In a large study, the relative risk of thrombotic strokes has been shown to range from 3 for normotensive users to 14 for users with severe hypertension. The relative risk of hemorrhagic stroke is reported to be 1.2 for non-smokers who used oral contraceptives, 2.6 for smokers who did not use oral contraceptives, 7.6 for smokers who used oral contraceptives, 1.8 for normotensive users and 25.7 for users with severe hypertension. The attributable risk is also greater in older women. d. Dose-related risk of vascular disease from oral contraceptives. A positive association has been observed between the amount of estrogen and progestogen in oral contraceptives and the risk of vascular disease. A decline in serum high density lipoproteins (HDL) has been reported with many progestational agents. A decline in serum high density lipoproteins has been associated with an increased incidence of ischemic heart disease. Because estrogens increase HDL cholesterol, the net effect of an oral contraceptive depends on a balance achieved between doses of estrogen and progestogen and the activity of the progestogen used in the contraceptive. The activity and amount of both hormones should be considered in the choice of an oral contraceptive. Minimizing exposure to estrogen and progestogen is in keeping with good principles of therapeutics. For any particular estrogen/progestogen combination, the dosage regimen prescribed should be one which contains the least amount of estrogen and progestogen that is compatible with a low failure rate and the needs of the individual patient. New acceptors of oral contraceptive agents should be started on preparations containing 0.035 mg or less of estrogen. e. Persistence of risk of vascular disease. There are two studies which have shown persistence of risk of vascular disease for ever-users of oral contraceptives. In a study in the United States, the risk of developing myocardial infarction after discontinuing oral contraceptives persists for at least 9 years for women 40-49 years who had used oral contraceptives for five or more years, but this increased risk was not demonstrated in other age groups. In another study in Great Britain, the risk of developing cerebrovascular disease persisted for at least 6 years after discontinuation of oral contraceptives, although excess risk was very small. However, both studies were performed with oral contraceptive formulations containing 50 micrograms or higher of estrogens. 2. ESTIMATES OF MORTALITY FROM CONTRACEPTIVE USE. One study gathered data from a variety of sources which have estimated the mortality rate associated with different methods of contraception at different ages. These estimates include the combined risk of death associated with contraceptive methods plus the risk attributable to pregnancy in the event of method failure. Each method of contraception has its specific benefits and risks. The study concluded that with the exception of oral contraceptive users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of birth control is low and below that associated with childbirth. However, smokers 35 and older and non-smokers 40 and older who use oral contraceptives have a significant increase in mortality higher than those using other methods of birth control. These facts must be weighed in conjunction with failure rates for other methods and the risk associated with subsequent pregnancy. 3. CARCINOMA OF THE REPRODUCTIVE ORGANS. Numerous epidemiological studies have been performed on the incidence of breast, endometrial, ovarian and cervical cancer in women using oral contraceptives. While there are conflicting reports most studies suggest that use of oral contraceptives is not associated with an overall increase in the risk of developing breast cancer. Some studies have reported an increased relative risk of developing breast cancer, particularly at a younger age. This increased relative risk appears to be related to duration of use. Some studies suggest that oral contraceptive use has been associated with an increase in the risk of cervical intraepithelial neoplasia in some populations of women. However, there continues to be controversy about the extent to which such findings may be due to differences in sexual behavior and other factors. 4. HEPATIC NEOPLASIA. Benign hepatic adenomas are associated with oral contraceptive use, although the incidence of benign tumors is rare in the United States. Indirect calculations have estimated the attributable risk to be in the range of 3.3 cases/100,000 for users, a risk that increases after four or more years of use especially with oral contraceptives of higher dose. Rupture of benign, hepatic adenomas may cause death through intra-abdominal hemorrhage. Studies from Britain have shown an increased risk of developing hepatocellular carcinoma in long-term (>8 years) oral contraceptive users. However, these cancers are rare in the U.S. and the attributable risk (the excess incidence) of liver cancers in oral contraceptive users approaches less than one per million users. 5. OCULAR LESIONS. There have been clinical case reports of retinal thrombosis associated with the use of oral contraceptives. Oral contraceptives should be discontinued if there is unexplained partial or complete loss of vision; onset of proptosis or diplopia, papilledema, or retinal vascular lesions. Appropriate diagnostic and therapeutic measures should be undertaken immediately. 6. ORAL CONTRACEPTIVE USE BEFORE OR DURING EARLY PREGNANCY. Extensive epidemiological studies have revealed no increased risk of birth defects in women who have used oral contraceptives prior to pregnancy. The majority of recent studies also do not indicate a teratogenic effect, particularly in so far as cardiac anomalies and limb reduction defects are concerned, when taken inadvertently during early pregnancy. The administration of oral contraceptives to induce withdrawal bleeding should not be used as a test for pregnancy. Oral contraceptives should not be used during pregnancy to treat threatened or habitual abortion. It is recommended that for any patient who has missed two consecutive periods pregnancy should be ruled out before continuing oral contraceptive use. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period. Oral contraceptive use should be discontinued until pregnancy is ruled out. 7. GALLBLADDER DISEASE. Earlier studies have reported an increased lifetime relative risk of gallbladder surgery in users of oral contraceptives and estrogens. More recent studies, however, have shown that the relative risk of developing gallbladder disease among oral contraceptive users may be minimal. The recent findings of minimal risk may be related to the use of oral contraceptive formulations containing lower hormonal doses of estrogens and progestogens. 8. CARBOHYDRATE AND LIPID METABOLIC EFFECTS. Oral contraceptives have been shown to cause a decrease in glucose tolerance in a significant percentage of users. This effect has been shown to be directly related to estrogen dose. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents. However, in the non-diabetic woman, oral contraceptives appear to have no effect on fasting blood glucose. Because of these demonstrated effects prediabetic and diabetic women in particular should be carefully monitored while taking oral contraceptives. A small proportion of women will have persistent hypertriglyceridemia while on the pill. As discussed earlier (see WARNINGS 1a and 1d), changes in serum triglycerides and lipoprotein levels have been reported in oral contraceptive users. 9. ELEVATED BLOOD PRESSURE. An increase in blood pressure has been reported in women taking oral contraceptives, and this increase is more likely in older oral contraceptive users and with extended duration of use. Data from the Royal College of General Practitioners and subsequent randomized trials have shown that the incidence of hypertension increases with increasing progestational activity. Women with a history of hypertension or hypertension-related diseases, or renal disease, should be encouraged to use another method of contraception. If women elect to use oral contraceptives, they should be monitored closely and if significant elevation of blood pressure occurs, oral contraceptives should be discontinued. For most women, elevated blood pressure will return to normal after stopping oral contraceptives, and there is no difference in the occurrence of hypertension between former and never users. 10. HEADACHE. The onset or exacerbation of migraine or development of headache with a new pattern which is recurrent, persistent or severe requires discontinuation of oral contraceptives and evaluation of the cause. 11. BLEEDING IRREGULARITIES. Breakthrough bleeding and spotting are sometimes encountered in patients on oral contraceptives, especially during the first three months of use. Non-hormonal causes should be considered and adequate diagnostic measures taken to rule out malignancy or pregnancy in the event of breakthrough bleeding as in the case of any abnormal vaginal bleeding. If pathology has been excluded, time or a change to another formulation may solve the problem. In the event of amenorrhea, pregnancy should be ruled out. Some women may encounter post-pill amenorrhea or oligomenorrhea, especially when such a condition was preexistent. 12. ECTOPIC PREGNANCY. Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. **PRECAUTIONS:** 1. PHYSICAL EXAMINATION AND FOLLOW UP. A complete medical history and physical examination should be taken prior to the initiation or reinstitution of oral contraceptives and at least annually during use of oral contraceptives. These physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including cervical cytology, and relevant laboratory tests. In case of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be conducted to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care. 2. LIPID DISORDERS. Women who are being treated for hyperlipidemias should be followed closely if they elect to use oral contraceptives. Some progestogens may elevate LDL levels and may render the control of hyperlipidemias more difficult. 3. LIVER FUNCTION. If jaundice develops in any woman receiving such drugs, the medication should be discontinued. Steroid hormones may be poorly metabolized in patients with impaired liver function. 4. FLUID RETENTION. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention. 5. EMOTIONAL DISORDERS. Women with a history of depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. 6. CONTACT LENSES. Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist. 7. DRUG INTERACTIONS. Reduced efficacy and increased incidence of breakthrough bleeding and menstrual irregularities have been associated with concomitant use of rifampin. A similar association, though less marked, has been suggested with barbiturates, phenylbutazone, phenytoin sodium, and possibly with griseofulvin, ampicillin and tetracyclines. 8. INTERACTIONS WITH LABORATORY TESTS. Certain endocrine and liver function tests and blood components may be affected by oral contraceptives: a. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3, increased norepinephrine-induced platelet aggregability. b. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or by radio-immunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. c. Other binding proteins may be elevated in serum. d. Sex-binding globulins are increased and result in elevated levels of total circulating sex steroids and corticoids; however, free or biologically active levels remain unchanged. e. Triglycerides may be increased. f. Glucose tolerance may be decreased. g. Serum folate levels may be depressed by oral contraceptive therapy. This may be of clinical significance if a woman becomes pregnant shortly after discontinuing oral contraceptives. 9. CARCINOGENESIS. See WARNINGS section. 10. PREGNANCY. Pregnancy Category X. See CONTRAINDICATIONS and WARNINGS sections. 11. NURSING MOTHERS. Small amounts of oral contraceptive steroids have been identified in the milk of nursing mothers and a few adverse effects on the child have been reported, including jaundice and breast enlargement. In addition, oral contraceptives given in the postpartum period may interfere with lactation by decreasing the quantity and quality of breast milk. If possible, the nursing mother should be advised not to use oral contraceptives but to use other forms of contraception until she has completely weaned her child. **INFORMATION FOR THE PATIENT: See Patient Package Insert.**

ADVERSE REACTIONS: An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS section). Thrombophlebitis and venous thrombosis with or without embolism. Arterial thromboembolism. Pulmonary embolism. Myocardial infarction. Cerebral hemorrhage. Cerebral thrombosis. Hypertension. Gallbladder disease. Hepatic adenomas or benign liver tumors. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea. Vomiting. Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding. Spotting. Change in menstrual flow. Amenorrhea. Temporary infertility after discontinuation of treatment. Edema. Melasma which may persist. Breast changes: tenderness, enlargement, secretion. Change in weight (increase or decrease). Change in cervical erosion and secretion. Diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives and the association has been neither confirmed nor refuted: Pre-menstrual syndrome. Cataracts. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiform. Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. Hemolytic uremic syndrome. Acne. Changes in libido. Colitis.

OVERDOSAGE: Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea and withdrawal bleeding may occur in females.

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Please see complete Prescribing Information, a brief summary of which is on the preceding page.

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STEROIDS FROM MENARCHE TO MENOPAUSE

Based on a symposium at the
XIII World Congress of
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Singapore
September 17, 1991

Chairman and Guest Editor
Daniel R. Mishell, Jr., MD

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Introduction

Daniel R. Mishell, Jr., MD

Los Angeles, California

An important symposium entitled "Steroids from Menarche to Menopause" was held on September 17, 1991, in Singapore at the XIII World Congress of Gynaecology and Obstetrics (International Federation of Gynecology and Obstetrics). Recent epidemiologic information on oral contraceptives and hormone replacement therapy, clinical considerations of hormone therapy, and future therapies were presented by an international faculty.

The first session was devoted to contraceptive steroids, and the second session dealt with the use of steroids after menopause. These topics were chosen because of their importance to women today and because of the great amount of misinformation that has been perpetuated about the dangers of these steroids.

Women in the United States try to avoid unwanted pregnancies for at least one third of their lives and for half of their reproductive lives, particularly between the time of the birth of their last wanted child (average age, 29 years) and the time of menopause. After menopause most women need estrogen replacement therapy for the remainder of their lives.

Oral contraceptive steroids, which have been used for 30 years, provide one of the most effective reversible methods of contraception. Although other options are available, most are not as effective and none are as popular in the United States. In addition, oral contraceptive steroids have many health benefits. Nevertheless, statistics show that a higher percentage of married women in the United States used oral contraceptives 18 years ago than in 1988. The formulations that were used 18 years ago contained higher doses of both estrogen and progestin and were associated with an increased risk of potential adverse effects. It is paradoxic that although safer oral contraceptive formulations are now available to prevent pregnancies, a smaller percentage of married women in the United States use them. The reason for this phenomenon is that most women have many concerns about these steroids. The main concerns that women have about oral contraceptives are as follows: (1) the potential increased risk of developing cancer, particularly breast cancer; (2) a potential adverse effect on the reproductive system that might lead to sterility and endanger future pregnancies; and (3) potential adverse metabolic effects that might lead to heart attacks, stroke, pulmonary emboli, and ultimately death. These concerns and fears are unwarranted. Data presented at this symposium show that the new low-dose steroids are safe and actually provide many noncontraceptive health benefits, including prevention of two lethal cancers (ovarian and endometrial).

The second half of the symposium was devoted to estrogen replacement after menopause. Female life expectancy in both developed and developing countries is increasing, mainly because of better obstetric care. Thus one third of the life of the average woman in the United States occurs after the menopause. This is a time of estrogen deficiency, which poses a wide range of problems. In the United States nearly 35 million women (30% of the female population) are in the postmenopausal age group; thus it is an important topic of public health concern.

The consequences of estrogen deficiency can be categorized as early problems (hot flushes, insomnia, depression, and other psychological symptoms); intermediate consequences, which occur approximately 5 years after the menopause (atrophy of the vagina, uterine prolapse, urgency and stress incontinence, and thinning of the skin); and the most severe consequences of estrogen deficiency, which begin about 10 years after menopause. These severe problems were addressed at this symposium: the development of osteoporotic fractures, which can cause immobilization and death, and the acceleration of atherosclerosis in the coronary arteries and cerebral vessels, which leads to increased incidences of heart attacks and stroke, the number one cause of death in women. These problems can often be delayed or even eliminated by the administration of estrogen.

The studies presented at this symposium demonstrate that steroids are beneficial both to younger women (because they prevent unwanted pregnancy and because of their many noncontraceptive health benefits) and to older women (because they prevent the problems associated with estrogen deficiency). Steroids used in oral contraceptives and estrogen replacement therapy are thus beneficial to a woman's health and longevity.

From the Department of Obstetrics and Gynecology, School of Medicine, University of Southern California.

6/0/37618

The safety of oral contraceptives: Epidemiologic insights from the first 30 years

David A. Grimes, MD
Los Angeles, California

Because oral contraceptives are used by tens of millions of healthy women, their safety for short-term and long-term use is an important issue that has been examined in a large number of epidemiologic studies. These studies have become more rigorous and have increased in size and analytic sophistication over the years. Although breast cancer remains the most important safety concern, the bulk of recent data suggests that oral contraceptives have no overall impact on a woman's risk of developing this disease. The results are less clear on the risk of cervical cancer and its precursors because of methodologic problems. However, the newer oral contraceptive formulations no longer appear to be associated with an increased risk of myocardial infarction or stroke. (AM J OBSTET GYNECOL 1992;166:1950-4.)

Key words: Epidemiologic studies, safety, breast cancer, ovarian cancer

Oral contraceptives are one of the most thoroughly studied medications ever used. Because tens of millions of healthy women worldwide have used oral contraceptives during the past 30 years and because some women have used them for extended lengths of time, the safety of short-term and long-term use of these preparations is a public health issue of great importance. Hence a large number of epidemiologic studies have been performed to address this concern. Oral contraceptives confer important health benefits unrelated to contraception which were unsuspected 30 years ago. Recent sophisticated studies have also alleviated concerns about potential cardiovascular and neoplastic risks associated with use of the pill. This review will highlight some of the important epidemiologic findings that concern the safety of oral contraceptives. It will describe the main types of epidemiologic studies that have been performed, summarize information on the established health benefits of oral contraceptives, and comment on several putative risks and lingering concerns.

Types of epidemiologic studies

All epidemiologic studies can be portrayed in a hierarchy of scientific value. The main types of studies can be divided into two categories: observational and experimental. In an observational study the investigator merely observes what happens in clinical practice. In contrast, in an experimental study the investigator intervenes by assigning treatments to subjects.

Observational studies can be further divided into descriptive and analytic studies. Descriptive studies include case reports and case-series reports (several cases in a single report). Descriptive studies, which are often performed to characterize a new disease or persons with a disease, have no comparison group; thus they cannot be used to test hypotheses or to draw conclusions about causation. Their greatest scientific usefulness may be to generate hypotheses that can then be tested in more rigorous studies. An example would be the case-series report that noted the appearance of functional ovarian cysts in women who use multiphasic oral contraceptives.[1]

Analytic studies can be divided into two types: cohort and case-control. In the former women are followed up from the time of exposure (e.g., use of contraception) to outcome (e.g., myocardial infarction). Advantages of cohort studies include the ability to calculate incidence rates, relative risks, and attributable risks. Examples of cohort studies include the Royal College of General Practitioners' study[2] and the Oxford/Family Planning Association study[3] in the United Kingdom and the Nurses' Health Study[4] in the United States. In these prospective studies thousands of women are being followed up from the time of exposure to oral contraceptives.

In contrast to cohort studies, case-control studies look backward in time. The investigator begins with an out-

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come (e.g., myocardial infarction) and looks back in time for exposure (e.g., type of contraception used). Case-control studies have contributed much knowledge about rare events and events that take a long time to develop (e.g., cancer). Unlike cohort studies, however, case-control studies do not enable investigators to calculate incidence rates, relative risks, or attributable risks. Instead investigators calculate odds ratios, which provide a good proxy for relative risks for infrequent diseases. An example of this type of study is the World Health Organization study[5] of the potential association between liver cancer and use of oral contraceptives.

The randomized, controlled trial is clinical medicine's closest approximation to the laboratory scientist's experiment. Use of randomized, controlled trials is the only way to avoid the biases that can undermine analytic studies: selection bias, ascertainment bias, and confounding. However, these trials are often complex and expensive to conduct. Because they rely on volunteers to act as subjects, the results may not be readily extrapolated to broad populations of women. In addition, this type of research poses unique ethical problems. Examples of randomized, controlled trials would be those that compare metabolic effects of different formulations of oral contraceptives.[6]

Established health benefits

Oral contraceptives may be the only prescription drug that increases life expectancy.[7] This benefit is derived from the oral contraceptives' powerful protection against several life-threatening diseases. The most important noncontraceptive health benefit is protection against ovarian cancer. In many developed countries this is the leading cause of death from gynecologic malignancy. Women who use oral contraceptives for as little as 6 months gain significant protection against ovarian cancer.[8] Results from studies around the world have been strikingly consistent; the risk of epithelial cancers of the ovary is reduced by approximately 40%.[9] In the largest case-control study of this phenomenon[8] protection against ovarian cancer lasted a minimum of 15 years after discontinuation of oral contraceptives. The longer a woman used oral contraceptives, the greater the protection gained; after 12 years of use, the risk of developing ovarian cancer was reduced by about 80%.

Protection against endometrial cancer is of the same order of magnitude: around 50%.[9] As is the case with ovarian cancer the protection against endometrial cancer persists for a minimum of 15 years after the last use of oral contraceptives.[10] The protection provided is greatest for those women who are at high risk, namely, nulliparous women. With increasing parity the protection moderates. All three main histologic types of endometrial cancer are reduced in frequency by use of oral contraceptives.[10]

The third life-threatening disease that is reduced in frequency by use of oral contraceptives is pelvic inflammatory disease. Women who take oral contraceptives have only half the risk of being hospitalized for this serious infection compared with women who do not use oral contraceptives.[11] In addition, women who develop pelvic inflammatory disease while they take oral contraceptives tend to have milder infections as judged by laparoscopy.[12] Whether this effect is mediated through changes in cervical mucus, decreased retrograde menstruation, decreased amount of monthly uterine bleeding, or other factors is unknown. Nevertheless the protection is strong and clinically important.

Ectopic pregnancy is the fourth potentially fatal condition that is reduced in frequency by use of oral contraceptives. Women who use oral contraceptives have approximately 90% fewer ectopic pregnancies than women who do not use contraceptives.[13] Without ovulation pregnancy should be impossible in any site.

Four conditions that impair quality of life are also reduced in frequency by use of oral contraceptives. These include iron deficiency anemia, primary dysmenorrhea, functional ovarian cysts, and benign breast disease.[13] The benefit related to this protection is large on an international scale. An alleged increase in the risk of developing functional ovarian cysts among women who do not use multiphasic pills[1] has not been corroborated by national statistics.[14]

In addition, four other protective effects of oral contraceptives have been suggested, although they have not been well established. These include protection against leiomyomata uteri, osteoporosis, toxic shock syndrome, and rheumatoid arthritis. Duration-related protection against leiomyomata was observed in the Oxford/Family Planning Association study.[15] Because this report challenges traditional thinking about the association between oral contraceptives and leiomyomata, this finding requires corroboration. Evidence is controversial about the magnitude and duration of protection against osteoporosis,[16] if it exists. A recent report[17] has found that oral contraceptives provide powerful protection against osteoporosis with longer use affording greater protection. The protection against toxic shock syndrome observed in a number of studies[18] appears to have been mediated through use of less absorbent tampons by women who used oral contraceptives.[13] Evidence remains divided as to protection provided by use of oral contraceptives against rheumatoid arthritis.[19] Alternatively, use of the pill may modify the course of the disease and prevent severe forms from developing.[20]

Potential risks

The single most worrisome question about the safety of oral contraceptive use is its potential effect on the development of breast cancer. During the past three decades many large studies found no overall effect. Authoritative reviews[21] of these studies, including two meta-analyses,[9, 22] have provided a reassuring picture. The aggregate relative risk of developing breast cancer is close to 1.0, with a very narrow 95% confidence interval. However, subgroup analyses have identified increased risks in certain populations, such as women aged 30 to 34 years or those with one child.[2] In general these findings have been inconsistent. In addition, a large case-control study[23] in the United Kingdom found a duration-related increase in the risk of developing breast cancer among young women who had used oral contraceptives, although the authors noted no corroboration from vital statistics of that nation.

In an attempt to replicate these findings, investigators in the United States reanalyzed the world's largest case-control study of this question.[24] Among nulliparous women they noted a suggestion of an increase in risk of developing breast cancer with increasing use of oral contraceptives by young women, but the opposite trend was discovered among older women.[25] This suggests that long-term use of oral contraceptives may promote breast cancer; that is, it may push forward in time the age at which breast cancer is diagnosed in women who are destined to develop the disease without having any net effect on a woman's lifetime risk.

Cervical cancer is a leading cause of cancer deaths in many developing countries. A large number of epidemiologic studies[9] have examined the potential association between use of oral contraceptives and this malignancy. The data are conflicting. In recent years several studies have reported an increased risk of developing cervical cancer among long-term users of oral contraceptives, for example, after 5 years of use.[25] It cannot be determined whether this reflects a causal relationship, because the study of cervical cancer poses serious methodologic challenges. For example, diagnostic bias is often a problem because women who use oral contraceptives are more likely to have regular cytologic screening than other women. The confounding effects of sexual behavior and cigarette smoking have not been adequately addressed in many studies. Nevertheless, it appears prudent to recommend that women who take oral contraceptives have cytologic screening a minimum of one time each year.

Liver cancer is another malignancy of great importance in the developing world. During the 1980s three case-control studies[9] from the United States and United Kingdom reported an increased risk of developing liver cancer among women who used oral contraceptives. However, a large case-control study[5] performed in developing countries by the World Health Organization found no increase in the risk of developing liver cancer with increasing duration of oral contraceptive use. Moreover, in developed countries no change in mortality rates among women from liver cancer has occurred despite extensive use of oral contraceptives during the past three decades.

Cardiovascular disease is the leading cause of death among women in many parts of the world. Whereas early epidemiologic studies[9] of high-dose oral contraceptives found significantly increased risks of developing cardiovascular disease among users of the pill, several trends have led to a reassessment of that risk. First, studies have become more sophisticated and now take into account the potentially confounding effects of cigarette smoking. Second, in recent years women have been evaluated more carefully before they begin to take oral contraceptives, and those women who have serious underlying medical problems and older women who smoke have been less likely to use oral contraceptives. Third, the formulations of oral contraceptives have changed dramatically; current formulations have a three- to fourfold decrease in estrogen dose and a 10-fold decrease in progestin dose compared with the formulations initially prescribed. Hence the findings of early epidemiologic studies are likely irrelevant to today's preparations.

Use of oral contraceptives by healthy women who do not smoke does not appear to be associated with an increased risk of either myocardial infarction or stroke. Data from the United States[27, 28] and the United Kingdom[3] have documented no increase in the risk of death from cardiovascular disease among contemporary users of oral contraceptives. Data from the Oxford/Family Planning Association study[29] also support the hypothesis that oral contraceptives that contain less than 50 μg of estrogen are safer than higher-dose formulations.

Some evidence suggests that the pathophysiology of myocardial infarction among women who used oral contraceptives in earlier studies was thrombosis. First, most studies, including the large Nurses' Health Study[4] from the United States, have found no increased risk of myocardial infarction among former users of oral contraceptives. If atherosclerosis were the cause then the risk should remain elevated after discontinuation of oral contraceptives. Corroborating evidence comes from autopsy studies of women who have died of myocardial infarction.[30] Second, there does not appear to be a duration-related effect between length of oral contraceptive use and risk of infarction.[28] This also argues against deposition of plaque as the pathophysiology. Third, studies performed in monkeys have demonstrated significant protection against the development of coronary atherosclerosis in animals who were given high progestin-dose combination oral contraceptives

that altered their lipid levels adversely.[31] This suggests that the estrogen in oral contraceptives may have a direct protective effect on the wall of the coronary vessels analogous to the protection against coronary artery disease provided by estrogen replacement therapy given to postmenopausal women.

The damaging effect of smoking has been clarified in recent years. In the Royal College of General Practitioners' study[32] the risk of myocardial infarction has been analyzed by amount of smoking and use of oral contraceptives. Current users of oral contraceptives who did not smoke had no increased risk of infarction. Current users of oral contraceptives who smoked fewer than 15 cigarettes a day had more than a threefold increase in risk. On the other hand, current users of oral contraceptives who smoked 15 or more cigarettes a day had a 21-fold increase in risk, a powerful synergistic effect. The consensus is clear: oral contraceptives are safe, and smoking is dangerous. It has been wryly noted that a strong case can be made on public health grounds for making oral contraceptives available over the counter and cigarettes available on prescription only.

In addition to the risk of developing cancer and cardiovascular disease, a third concern women have about oral contraceptives is potential infertility after discontinuation of the pills. In the Oxford/Family Planning Association study[33] the resumption of fertility after discontinuing oral contraceptives was similar to resumption of fertility after discontinuation of other methods of contraception, although a transient delay was found. A recent analysis[34] of this question in the United States also confirmed a temporary delay of about 2 months on average in return of fertility. This delay was dose related. Lower-dose pills were associated with a shorter delay than were higher-dose pills.

The current status

In summary, recent, large, and sophisticated epidemiologic studies around the world have provided an encouraging assessment of oral contraceptives. Oral contraceptives protect against the development of ovarian and endometrial cancers, pelvic inflammatory disease, and ectopic pregnancy; they also protect against the development of iron deficiency anemia, primary dysmenorrhea, functional ovarian cysts, and benign breast disease. Most studies show no overall effect on the risk of developing breast cancer. The potential effect of long-term oral contraceptive use on breast cancer remains controversial and will require further evaluation. Although several studies have suggested an increased risk of developing cervical neoplasia with long-term oral contraceptive use, methodologic difficulties preclude drawing conclusions about causality. Nevertheless, women who use oral contraceptives should have routine cytologic screening. Several lines of evidence argue against an association of oral contraceptive use with liver cancer.

The increased risk of developing cardiovascular disease among women who use oral contraceptives in older studies does not appear to relate to contemporary oral contraceptive use. In recent studies neither current nor previous oral contraceptive use has been shown to increase the risk of myocardial infarction or stroke. Atherosclerosis does not appear to be an important cause of myocardial infarction in women who use oral contraceptives. Finally, a transient delay in return of fertility can occur after use of oral contraceptives is discontinued, although the effect is lessened in women who use low-dose oral contraceptives.

When future medical historians look back on the twentieth century, the development of oral contraceptives will stand out as a landmark medical achievement. Like antibiotics, oral contraceptives are life-saving medications. Their use worldwide has averted much morbidity and mortality related to unintended pregnancy. Moreover, when used by healthy women, the noncontraceptive benefits outweigh the risks involved. To paraphrase William Foege, MD, former director of the Centers for Disease Control, "This may be the most noble medicine of all . . . the anonymous gift of health."

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Clinical and metabolic considerations of long-term oral contraceptive use

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Newer lower dose formulations are associated with an improved cardiovascular disease risk marker profile, which supports their use for longer periods and among older women. Epidemiologic studies of the newer formulations are limited. Without clinical information, an evaluation of the effects of more recent formulations on metabolic risk markers for cardiovascular disease is useful. In a large cross-sectional study, a reduction in the progestin dose and use of alternative progestins substantially reduced the proportion of oral contraceptive users with values associated with an increased risk of cardiovascular disease. No progression in metabolic changes was found by analyzing the effect of the duration of oral contraceptive use. The user's age interacted positively with the oral contraceptive–induced increase in serum triglyceride levels, but there was no interaction of age with the oral contraceptive's effect on oral glucose tolerance, glucose and insulin responses, low-density lipoprotein cholesterol, or high-density lipoprotein subfraction 2 cholesterol levels. (Am J Obstet Gynecol 1992;166:1955-63.)

Key words: Cardiovascular disease, progestins, metabolic risk markers.

With the introduction of low-dose oral contraceptives and more selective progestins such as desogestrel, gestodene, and norgestimate, the possibility now exists that duration of oral contraceptive use may be prolonged and that oral contraceptive use may be extended to women older than age 35 years. Such use raises specific issues of oral contraceptive safety. Questions to be addressed include whether extending the duration of continuous oral contraceptive use would result in a commensurate increase in any possible risks and whether older women are at increased risk for adverse effects associated with oral contraceptive use compared with their younger counterparts.

Recommendations against the use of combination oral contraceptives in women older than 35 years are based on findings from some epidemiologic studies performed during the 1970s, principally the Royal College of General Practitioners' Oral Contraceptive Study.[1, 2] This study found that cardiovascular disease was the principal adverse consequence of oral contraceptive use, with risk associated in women older than 35 years who were smokers. Most oral contraceptive formulations included in this and other epidemiologic studies contained 50 μg or more of estrogen. Currently support exists for extending combined oral contraceptive use in older women based on the recognition that, as a result of the introduction of new, lower dose formulations, the risks are likely to have been significantly reduced since the original studies were performed.

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Long-term oral contraceptive use and cardiovascular disease

Studies of the older medium- and high-dose formulations still provide our only reliable information on long-term oral contraceptive safety and should be taken into account when considering issues related to currently used formulations. Not all studies examined the interactions of duration of use and age of user with risk of cardiovascular disease. Those studies[3-9] that showed a positive association between current oral contraceptive use and cardiovascular disease and that specifically addressed interactions with age and duration are listed in Table I.

Effects of duration of oral contraceptive use. In the case-control study of mortality from myocardial infarction by Mann et al.,[3, 4] most patients who took oral contraceptives had used them for 2 or more years. Other case-control studies have provided no evidence of an interaction of duration of use with cardiovascular disease risks associated with current oral contraceptive use. Likewise, prospective studies have provided little evidence of an effect of duration or use. The 1977 Royal College of General Practitioners' Oral Contraceptive Study described an increased risk of cardiovascular disease with increasing duration of use,[8] but this was not confirmed in subsequent reports.[1, 2] There was also no evidence of increased risk of cardiovascular disease with prolonged duration of oral contraceptive use in cases in the Oxford/Family Planning Association Cohort.[9]

Effects of age of oral contraceptive user. The case-control study of mortality from myocardial infarction of Mann et al.[3, 4] found increased risk of coronary heart

Table I. Epidemiologic studies that showed an increased risk of ischemic heart disease in current oral contraceptive users and examined the effects of duration of oral contraceptive use and age of the user

Study	*Duration effect*	*Age effect*
Case-control studies		
Mann and Inman, 1975,[3] and Mann et al., 1976[4]	Yes	Yes
Shapiro et al., 1979[5]	No	No
Slone et al., 1981[6]	No	No
Rosenberg et al., 1980[7]	No	No
Prospective studies		
Royal College of General Practitioners' Oral Contraceptive Study 1977[8]	Yes	Yes*
Royal College of General Practitioners' Oral Contraceptive Study 1981[1]	No	Yes*
Royal College of General Practitioners' Oral Contraceptive Study 1983[2]	No	Yes†
Vessey et al., 1989[9]	No	—

*All circulatory diseases.
†All arterial diseases.

disease in women aged 40 to 44 years compared with their younger counterparts. However, the case-control study of survivors of a myocardial infarction of the Boston University Drug Epidemiology Unit[5, 6] found no increased risk in older compared with younger oral contraceptive users. The concern about a correlation between the user's age and the risk associated with oral contraceptive use stems largely from the findings of the Royal College of General Practitioners' Oral Contraceptive Study. In the 1977 report on this study, a rate ratio of 2.0 was found for mortality from vascular disease in women aged 25 to 34 years who had ever used oral contraceptives, whereas the corresponding rate for users aged 35 to 44 years was 4.5.[8] In the 1981 report on this study, the corresponding figures were 1.6 and 3.3, respectively, among nonsmokers and 3.4 and 4.2 among smokers.[1] In the 1983 report on arterial disease, the corresponding figures were 1.5 and 1.8, respectively, among nonsmokers and 1.6 and 3.1 among smokers.[2] In the Oxford/Family Planning Association Study most cases of vascular disease were in women older than 35 years of age.[10]

Effects of previous oral contraceptive use. An important question in long-term oral contraceptive use is whether there is an increased risk of cardiovascular disease in women who are not current users but who have used oral contraceptives in the past. None of the case-control studies that demonstrated an increased risk in current users and in which risk in a past-user group was examined showed an increased risk of cardiovascular disease in previous users. However, only one[6] of these studies was specifically designed to investigate the effects of past use.

Among prospective studies, a past-use effect was apparent for cerebrovascular disease but not for ischemic heart disease in the Royal College of General Practitioners' Oral Contraceptive Study.[1] The Nurses' Health Study[11] found some evidence of an increased risk of cerebrovascular disease with increasing duration of previous oral contraceptive use, although this relationship was not significant. In the Oxford/Family Planning Association Study,[9] increased risk of mortality from ischemic heart disease was concentrated among past users, but the authors noted that these women had generally stopped using oral contraceptives because they developed side effects.

Three studies have addressed the issue of a past-use effect in detail. In a case-control study from the Boston University Drug Epidemiology Unit, Slone et al.[6] noted a significant trend in increasing risk of myocardial infarction with duration of previous high-dose oral contraceptive use in women aged 40 to 49 years. This finding was not confirmed by the Nurses' Health Study[11] or by a subsequent study by Rosenberg et al.[12] The design of the last study reproduced that of the earlier study by Slone et al., and they concluded that the absence of a past-use effect could have reflected the change toward lower dose oral contraceptives and more selective prescribing.

Metabolic risk markers for cardiovascular disease in long-term oral contraceptive use

Information on cardiovascular disease risks associated with the use of oral contraceptives containing less than 50 μg of estrogen is limited, although the available data suggest that risks may have been reduced.[13, 14] Little is known of how the user's age or duration of use interact with any risks associated with these low-dose formulations. The risks associated with low-dose oral contraceptives containing the newly synthesized progestins must also be evaluated.

The difficulties in performing effective epidemiologic studies on the effects of oral contraceptive use and the length of time needed before potential side effects can be confirmed have favored the use of alternative methods of evaluating long-term oral contraceptive safety. The most important method has been the investigation of oral contraceptive–induced bio-

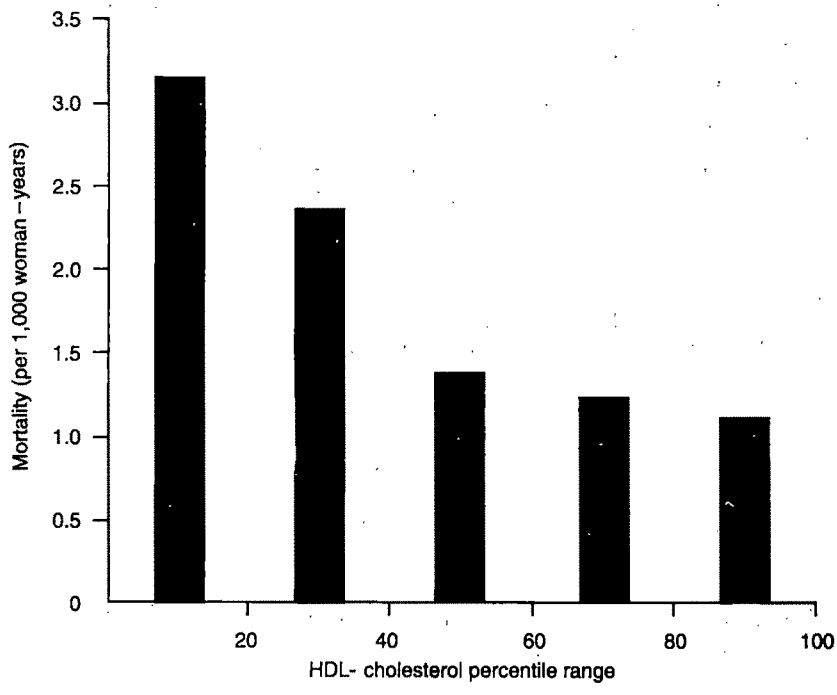


Fig. 1. Mortality from cardiovascular disease by HDL-cholesterol percentile in 3417 women. (Modified from Jacobs DJ, Mebane I, Bangdiwala S, Criqui M, Tyroler H. High density lipoprotein cholesterol as a predictor of cardiovascular disease mortality in men and women: the follow-up study of the Lipid Research Clinics Prevalence Study. Am J Epidemiol 1990;131:32-47.)

chemical alterations, primarily in lipid and carbohydrate metabolism. These disturbances resemble those seen in individuals at increased risk of cardiovascular disease. For example, in Fig. 1, data from the Lipid Research Clinics Prevalence Study show that in passing from the top to the bottom quintile of high-density lipoprotein (HDL) cholesterol concentrations, there is a threefold increase in cardiovascular disease risk in women.[15] Variation in the HDL subfraction 2 (HDL_2) appears to be particularly important with regard to cardiovascular disease risk.[16] Using data from our recent large cross-sectional study,[17] we have derived HDL_2-cholesterol concentration quintile ranges for women who do not take oral contraceptives ($n = 418$) and determined the prevalence of women taking a commonly used combination oral contraceptive formulation (150 μg of levonorgestrel + 30 μg of ethinyl estradiol) occurring in each quintile. Prevalence ratios in each HDL_2-cholesterol quintile are shown in Fig. 2. There was a fourfold greater incidence of observed than expected oral contraceptive users in the lowest HDL_2-cholesterol quintile, the quintile associated with the greatest risk of cardiovascular disease.

The plasma insulin concentration also provides an indicator of cardiovascular disease risk. Elevated insulin concentrations are associated with potentially atherogenic changes in the arterial wall,[18] elevated blood pressure,[19] and reduced fibrinolytic activity.[20] Hyperinsulinemia is also associated with insulin resistance, which was recently identified as a common factor in several biochemical disturbances seen in persons at increased risk of cardiovascular disease.[21] These biochemical disturbances include impaired glucose tolerance, low HDL-cholesterol concentrations, high low-density lipoprotein (LDL) cholesterol concentrations, hypertension, and hypertriglyceridemia. Epidemiologic studies of the association between insulin concentrations and cardiovascular disease are limited, and reliable information has only been obtained from studies in men.[22, 23] Nevertheless, as shown in Fig. 3, it is likely that a substantial increase in cardiovascular disease risk will occur with increasing insulin concentrations. In the Helsinki Policemen Study, the incidence of coronary heart disease increased fivefold in the 10% of individuals with the highest insulin concentrations during an oral glucose tolerance test (OGTT) compared with those in the lowest quintiles of insulin concentration.[23] We defined comparable percentile ranges for OGTT insulin response in 346 women who did not take oral contraceptives and determined the prevalence of women taking the low, fixed-dose levonorgestrel combination in each range (Fig. 4). A fourfold increase occurred in the proportion of oral contraceptive users in the top tenth percentile, the percentile associated with the greatest increase in coronary heart disease risk.

Whether oral contraceptive–induced metabolic disturbances affect the incidence of cardiovascular disease in women who use oral contraceptives is unknown. Al-

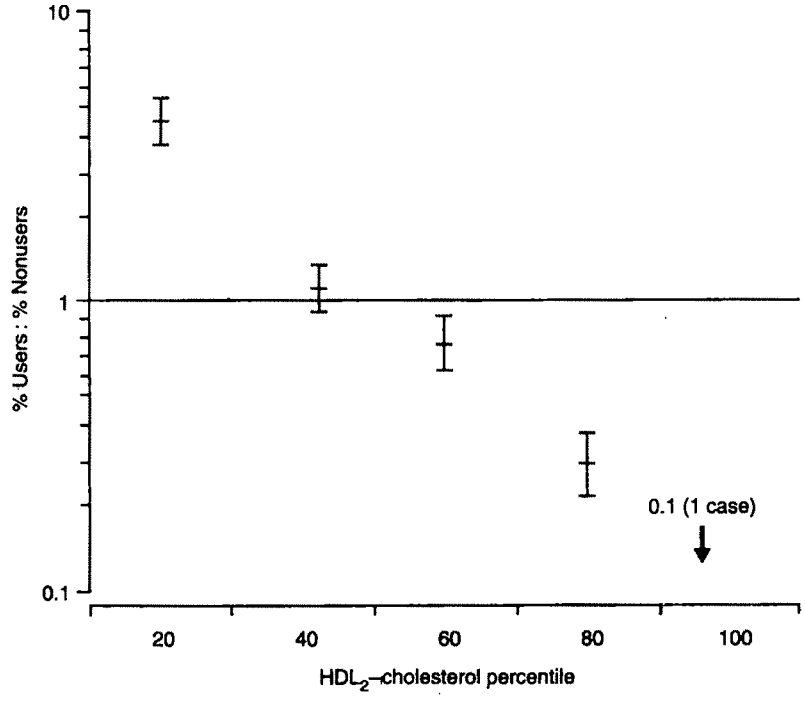


Fig. 2. Prevalence ratios and confidence intervals for 286 users of an oral contraceptive formulation (30 μg of ethinyl estradiol ± 150 μg of levonorgestrel) in HDL_2 cholesterol concentration percentile ranges defined in 418 nonuser control subjects.

though the information from epidemiologic studies is limited, it is advisable to minimize these disturbances if possible. This can be achieved by reducing the estrogen and progestin doses. It may also be possible to reduce the metabolic impact by using alternative oral contraceptive steroids. This is the underlying principle for the development and introduction of the new generation of progestins, which include desogestrel, gestodene, and norgestimate. These progestins have been selected for their lack of androgenic or antiestrogenic properties, which leads to a reduction in potentially adverse biochemical effects, particularly with regard to HDL cholesterol.

In our cross-sectional study, variation in HDL-cholesterol concentrations was primarily caused by variation in the HDL_2 subfraction.[17] The percentage differences between mean values for both HDL-cholesterol and HDL_2-cholesterol in nonuser control subjects and oral contraceptive users, who were taking one of seven different combined formulations, demonstrated a marked effect of the reduction in progestin dose, particularly with the levonorgestrel formulations. The effect of using alternative progestins was also apparent: lower mean values were generally found among women who used the low-dose norethindrone and desogestrel combinations. These findings were also the case when OGTT insulin responses were compared between the different formulations. We further analyzed the frequency distributions for these variables in the different oral contraceptive groups compared with the distribution in nonusers. The results indicate that reductions in metabolic impact apparent in the differences between means are also apparent in the differences in the proportion of outlying values.[24] Thus the lower HDL_2-cholesterol concentrations in users of fixed-dose levonorgestrel-containing formulations compared with nonusers were the result of an increase in the proportion of abnormally low HDL_2-cholesterol concentrations. As indicated earlier, such outlying values may be associated with an increased risk of coronary heart disease.

These observations clearly demonstrate that a reduction in the steroid dose of oral contraceptives and use of alternative progestins can markedly reduce potentially adverse metabolic changes, even among users of low-dose formulations. Thus extending oral contraceptive use among older women and prescribing oral contraceptives for longer periods may be appropriate.

Just as it was possible to assess interactions of duration of oral contraceptive use and age of the user with

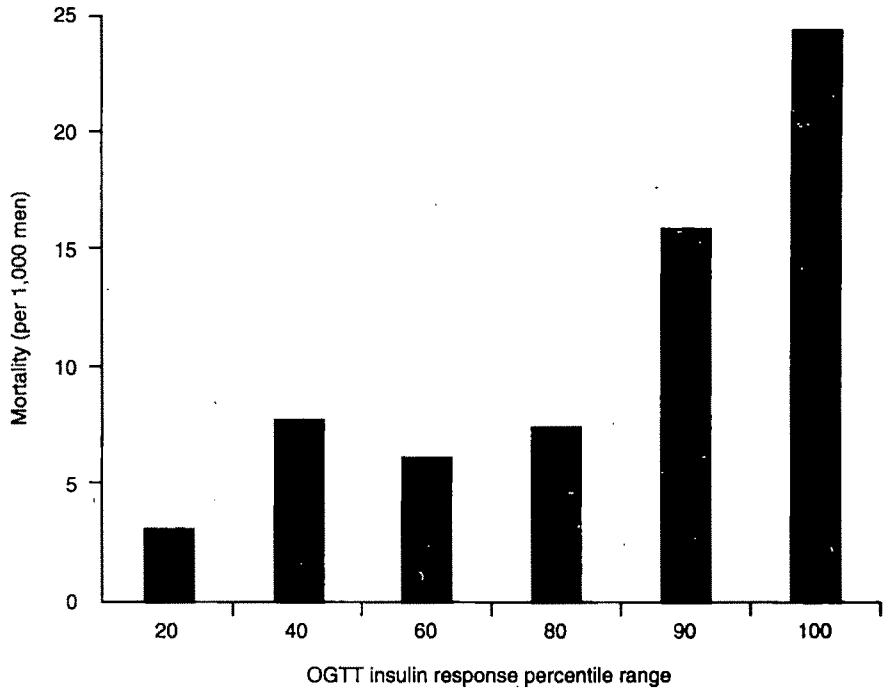


Fig. 3. Mortality from coronary heart disease OGTT insulin area percentile in 982 men. (From Pyörälä K, et al. Acta Med Scand 1985;701(suppl):38-52.)

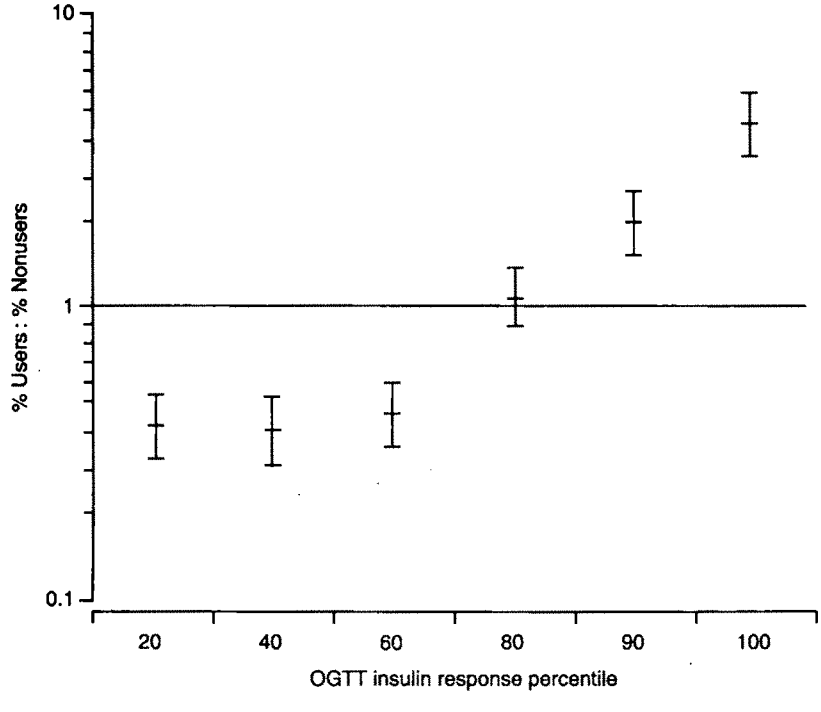


Fig. 4. OGTT incremental insulin area prevalence ratios and confidence intervals for 215 users of an oral contraceptive formulation (30 μg of ethinyl estradiol ± 150 μg of levonorgestrel) in percentile ranges defined in 346 nonuser control subjects.

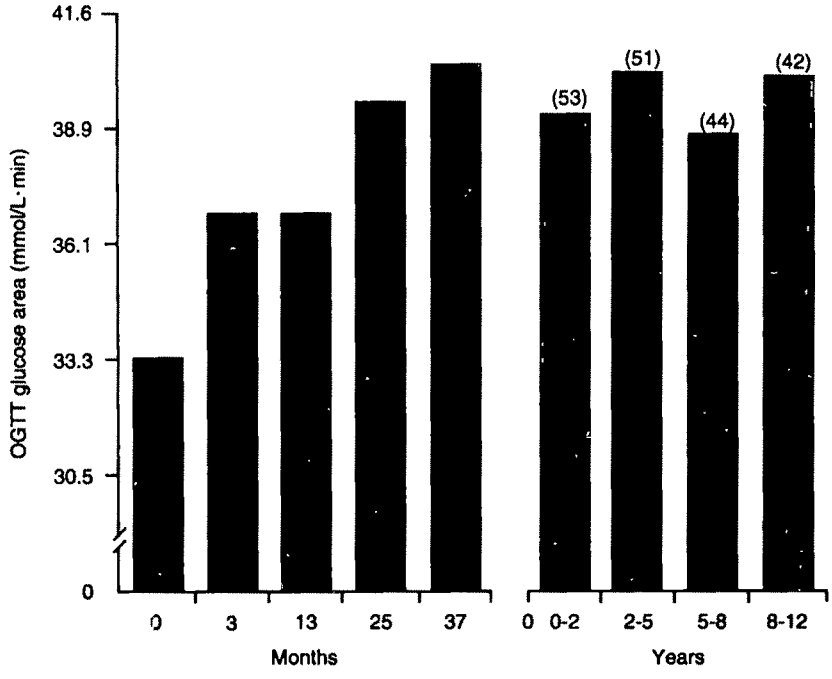


Fig. 5. OGTT glucose area in 17 oral contraceptive users followed up for 37 months *(left)* and in groups of oral contraceptive users (number in parentheses) who have been taking oral contraceptives for up to 2, 5, 8, and 12 years *(right)*. (Data from Wynn V. AM J OBSTET GYNECOL 1982;142:739-46.)

risk of cardiovascular disease in early epidemiologic studies, it should also be possible to assess the interactions of these same two factors with disturbances in metabolic risk markers. Few metabolic studies have addressed these issues, but with the large body of data from our recent cross-sectional study, an analysis of the effects of these factors has been possible.

Effects of duration of oral contraceptive use. Longitudinal studies of the metabolic effects of oral contraceptives have generally been short (<18 months) and have not included sufficient numbers of patients to distinguish significant effects from the considerable intraindividual variability that exists in metabolic variables. An exception was the study by Wynn,[25] who found that in a group of women taking a commonly used combination oral contraceptive (150 μg of levonorgestrel + 30 μg of ethinyl estradiol), deterioration in glucose tolerance progressed with time for up to 3 years. In this study 173 women were tested before and 3 months after they took the oral contraceptive; 86 of these women were tested after 15 months, 39 after 25 months, and 17 after 37 months. Results for the 17 women in the longest series are summarized in Fig. 5, which shows the progressive increase in the area under the OGTT glucose concentration profile with increasing duration of oral contraceptive use. After the initial oral contraceptive–induced elevation in the OGTT insulin response, there was no such progressive change. The initial progression in deterioration in glucose tolerance seen with this levonorgestrel formulation was not seen with a norethindrone-containing, low-estrogen dose formulation.[26]

In our cross-sectional study we further examined the effect of duration of oral contraceptive use in women who were current users of the same fixed-dose, levonorgestrel-containing formulation that was studied in the earlier longitudinal study (150 μg of levonorgestrel + 30 μg of ethinyl estradiol). We divided the users into approximately equal groups according to increasing duration of continuous oral contraceptive use. Mean OGTT glucose areas in women taking oral contraceptives from 3 months to 2 years, from 2 to 5 years, from 5 to 8 years, and from 8 to 12 years are shown in Fig. 5. Results have been standardized for several confounding variables, including age and percentage of ideal body weight. No continuing deterioration in glucose tolerance was seen from 2 to 12 years of use. Similar analyses for OGTT insulin area, fasting serum triglyceride levels, LDL cholesterol, and HDL_2 cholesterol (results not shown) showed no progression with increasing duration of oral contraceptive use.

Effects of age of oral contraceptive user. If the age of the oral contraceptive user is a factor in the degree of metabolic disturbance induced by an oral contraceptive, then a different relationship between age and metabolic variables will be seen in users and nonusers.

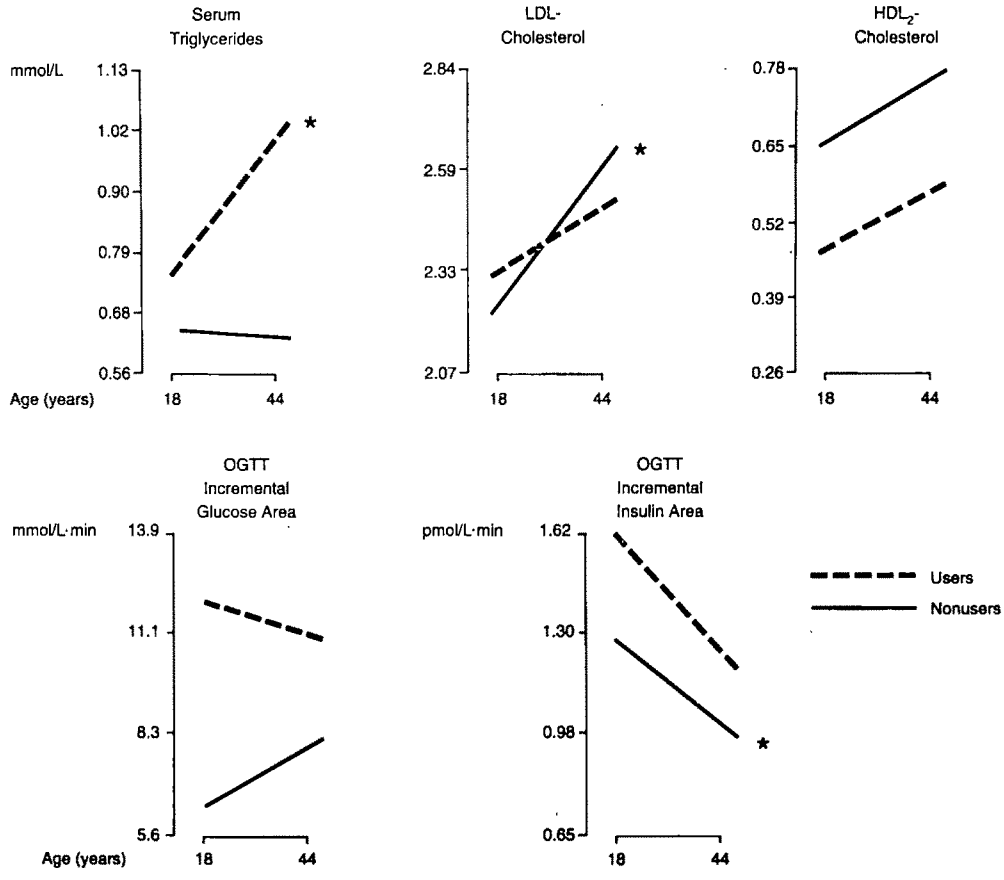


Fig. 6. Regression lines for age (18 to 44 years) with serum triglycerides, LDL and HDL_2 cholesterol and OGTT incremental glucose and insulin areas in nonusers (n = 418, lipids and lipoproteins; n = 296, glucose and insulin) and oral contraceptive users (n = 346, lipids and lipoproteins; n = 215, glucose and insulin). *Significant associations ($p < 0.05$.)

Table II. Age, percentage of ideal body weight, glucose area, OGTT insulin area, fasting serum triglycerides, LDL, and HDL_2 for women not currently using oral contraceptives who had never used them (never users) or who had used them in the past (former users)

Variables	*Never User (n = 67)*	*Former user (n = 209)*
Age (yr)	31.1 ± 7.9	32.3 ± 6.6
% Ideal body weight	100.8 ± 10.1	98.6 ± 9.5
OGTT glucose area (mmol/L · min)	35.8 ± 5.0	36.0 ± 5.5
OGTT insulin area (pmol/L · min)*	1629 − 587 + 917	1422 − 474 + 710†
Triglycerides (mmol/L)*	0.71 − 0.22 + 0.32	0.66 − 0.20 + 0.28
LDL (mmol/L)	2.49 ± 0.83	2.47 ± 0.70
HDL_2 (mmol/L)	0.64 ± 0.24	0.75 ± 0.31‡

Mean ± standard deviation.
*Logarithmically derived data.
†$p < 0.05$, ‡$p < 0.01$, by unpaired t test.

We addressed this issue by deriving the linear regression equations for age with fasting serum triglycerides, LDL and HDL_2 cholesterol, and OGTT glucose and insulin incremental areas with standardization for a number of confounding variables including current and previous cigarette smoking, percentage of ideal body weight, family histories of heart disease and diabetes, alcohol intake, and exercise habit. The analyses were performed for nonuser controls and for women taking the low, fixed-dose levonorgestrel monophasic combination (150 μg of levonorgestrel + 30 μg of ethinyl estradiol); this was the largest group we studied. The regression lines over the range of ages studied (18 to 44 years) are shown in Fig. 6. A marked interaction

was apparent between age and oral contraceptive use with regard to the oral contraceptive–induced rise in fasting serum triglyceride levels; triglyceride levels increased with age in oral contraceptive users but not in nonuser control subjects. However, with LDL cholesterol there was somewhat less of an effect of age in oral contraceptive users than in the nonusers, and there was no difference in interaction with age between users and nonusers with regard to HDL_2 cholesterol. There was also somewhat less of an effect of age in oral contraceptive users with regard to glucose tolerance and no difference between users and nonusers with regard to OGTT insulin response.

Effect of previous oral contraceptive use. In our cross-sectional study, 276 women who were not current oral contraceptive users claimed to have accurate recall of their previous contraceptive history. Of these, 67 had never used oral contraceptives and 209 had used oral contraceptives previously but had not taken oral contraceptives for at least 3 months (mean, 46.3 months). Metabolic variables were compared between never users and former users (Table II).

The only significant differences between never users and former users were in OGTT insulin response and HDL_2 cholesterol. With standardization for age and percentage of ideal body weight, the difference in insulin response was no longer significant, and with standardization for alcohol intake, the difference in HDL_2 cholesterol was no longer significant. Therefore there was no residual effect of previous oral contraceptive use on metabolic variables apparent in the group of former users we studied.

Conclusion

Epidemiologic studies of the older oral contraceptives with high and medium does of estrogen suggest that women older than age 35 years are at higher risk of cardiovascular disease from oral contraceptive use than their younger counterparts. Evidence for an effect of duration of oral contraceptive use or a past-use effect with these agents has not been confirmed.

We found no apparent progression in metabolic risk markers for cardiovascular disease beyond 2 years of continuous oral contraceptive use. There was no potentially adverse interaction between the age of the user and the duration of use and the oral contraceptive's effects on OGTT glucose and insulin areas, LDL cholesterol, and HDL_2 cholesterol. Older women who used oral contraceptives, however, appeared to have appreciably higher triglyceride concentrations than younger oral contraceptive users or nonusers of the same age. Furthermore, no effect of previous oral contraceptive use was apparent in former users.

These considerations support a cautious extension of oral contraceptive use among women older than 35 years of age. However, regular health checks and measurement of serum lipid levels would be desirable.

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The efficacy and tolerability of norgestimate/ethinyl estradiol (250 μg of norgestimate/35 μg of ethinyl estradiol): Results of an open, multicenter study of 59,701 women

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The efficacy and tolerability of a new oral contraceptive, norgestimate/ethinyl estradiol (250 μg of norgestimate/35 μg of ethinyl estradiol; Cilag GmbH Research, Sulzbach, Germany) were examined in an open-label study of 59,701 women who were evaluated during 342,348 menstrual cycles; 42,022 women completed the planned treatment regimen of six cycles. A use-efficacy (overall) Pearl index of 0.25 pregnancies per 100 woman-years was calculated based on 342,348 cycles. Tolerability was assessed for all women who completed six treatment cycles. Reductions in mean cycle length and duration of bleeding were noted; 32% of the women experienced reductions in the intensity of bleeding by the end of cycle 6. After six cycles of use, amenorrhea occurred in 1%, spotting in 4%, and breakthrough bleeding in 3% of the participating women. Treatment with norgestimate/ethinyl estradiol had minimal effects on weight, blood pressure, pulse, lipid metabolism, and blood glucose. Adverse effects (acne, nausea, or headaches) occurred at low frequencies and in many cases, were reduced compared with pretreatment levels. The results of this large-scale open trial were comparable with results from two other multicenter trials of the same formulation. (AM J OBSTET GYNECOL 1992;166:1963-8.)

Key words: Norgestimate, ethinyl estradiol, progestins, oral contraceptives

In recent years, much research has focused on the development of oral contraceptives with reduced hormone content.[1] Both the estrogen and progestin components of oral contraceptive formulations are thought to contribute to minor adverse effects, such as nausea, headache, and bleeding irregularities[1] and to more serious health problems, such as an increased risk of cardiovascular disease noted with high-dose formulations.[2-4]

Among the minor adverse effects, nausea and headache are generally linked to estrogen dose. With the introduction of oral contraceptive preparations with estrogen doses of 50 μg or less, the incidence of these adverse effects has generally been reduced.[1] However, breakthrough bleeding (BTB) and spotting, which appear to be determined by the estrogen/progestin ratio, have been more difficult to address.

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Most of the early studies suggested that the estrogen content of oral contraceptives was related to venous but not to arterial thromboembolic disease.[5] This finding led to investigation of the influence of the progestin component of oral contraceptives on various risk factors for occlusive arterial disease. In general, estrogen has a favorable effect on cholesterol fractions: it is associated with a reduction in serum concentration of low-density lipoprotein cholesterol and an increase in high-density lipoprotein cholesterol, as well as in the high-density lipoprotein subfraction, HDL_2. All progestins now in clinical use, however, oppose the effects of the estrogen component.[6]

Thus in recent years, one goal of oral contraceptive research has been the development of a highly selective progestin with specific and potent progestational activities and minimal or no androgenic effects.[6] Norgestimate (NGM), a progestin with marked progestational activity and a relative lack of androgenic activity, is promising in this regard.[7] Orally administered NGM is rapidly absorbed, reaches peak blood levels within 30 minutes to 2 hours, and has a plasma half-life of 6 to 14 hours. In the laboratory NGM has been extensively studied. NGM and its major metabolite 17-deacetyl norgestimate contribute to the biologic response.[8]

In previous clinical trials, the efficacy and safety of NGM/ethinyl estradiol (EE) (250 μg of NGM/35 μg of EE; Cilest, Cilag GmbH Research, Sulzbach, Germany; Ortho-Cyclen, R. W. Johnson Pharmaceutical Research Institute, Raritan, N.J.) have been established.[6, 9] The current open-label trial, which enrolled 59,701 women, for a planned treatment period of six cycles, was designed to generate additional data on the efficacy and tolerability of NGM/EE, with special emphasis on changes in bleeding and in metabolic parameters.

Methods

Study design. The trial was an open-label, noncomparative study with a large patient population. The planned length of active treatment was six cycles.

Patient selection. Women of childbearing age who were in good general health and had regular menstrual cycles of approximately 28 to 32 days' duration (including 3 to 5 days of bleeding) were eligible for entry. Exclusion criteria included: (1) the presence of vascular or hematologic disorders such as acute or chronic thromboembolism, cerebrovascular illness, myocardial infarction, or sickle cell anemia; (2) the presence of reproductive disorders such as amenorrhea, a history of herpes gestationis, or severe pruritus during pregnancy; (3) suspected or current pregnancy; and (4) general medical problems such as liver or gallbladder disease, cancer, or other metabolic derangements.

Data collection. Data gathered during the initial visit included age, height, and weight, as well as information on smoking habits, alcohol consumption, gynecologic history (including previous contraceptive use), family medical history, and the use of other concurrent medications.

During the study, information on the cycle (including cycle length, duration and intensity of bleeding, lack of bleeding during tablet-free interval, occurrence of spotting or BTB, dysmenorrhea, and other gynecologic findings) was recorded by each patient on her study calendar, which she brought with her to the checkup examinations after the third and sixth cycles.

Clinical variables measured during office visits before treatment and after the third and sixth cycle included blood pressure, pulse, and body weight. Concomitant symptoms, such as acne, nausea, breast tension, headaches, or other, were recorded by the patient on her study calendar. Reports on adverse symptoms were elicited by a questionnaire.

Laboratory variables, including serum cholesterol, serum triglyceride, and blood and urinary glucose levels, were evaluated in a subgroup of 3000 women before the study and after the third and sixth cycles.

Evaluation of data. The efficacy of NGM/EE was based on the computation of the overall Pearl index[10] for the entire study population and the related 95% confidence intervals, which were computed under the assumption of Poisson-distribution data.[11] Tolerability was evaluated by tabulating data on the menstrual cycle, adverse experiences, routine physical findings, and measurements of serum cholesterol, triglyceride, blood glucose, and urinary glucose levels for patients who completed cycle 6 of treatment with NGM/EE.

Results

Premature termination. During the study, 59,701 patients were enrolled by 1609 physicians. Treatment was discontinued prematurely in 6588 patients (11%). Terminations were at the physicians' request, at the patients' request, or were the result of a joint decision (Table I).

Patient characteristics. Among the 59,701 women who received NGM/EE, mean (± standard deviation) age was 24.0 ± 6.7 years, mean height was 166.4 ± 6.3 cm, mean weight was 61.2 ± 9.0 kg, and mean Broca index was 103.0% ± 23.3% (Broca index = body weight/0.9 × [height/100]). The mean (± standard deviation) age at menarche was 12.9 ± 1.3 years. Before they received NGM/EE, 88% of the women had a regular menstrual cycle (i.e., range, 24 to 32 days). Among women with regular cycles, the mean (± standard deviation) duration of the cycle was 28.0 ± 2.2 days, and the mean duration of bleeding was 4.9 ± 1.3 days. The intensity of the bleeding was designated as normal by 74% of the women, light by 13%, and heavy by 14%. Of the study subjects, 14% reported inter-

Table I. Persons responsible for decisions to withdraw from treatment, by cycle

Cycle	*Physician*	*Patient*	*Both*	*Unknown*	*Totals (%)*
1	87	300	59	70	516 (7.8)
2	91	428	62	54	635 (9.6)
3	397	1909	258	193	2757 (41.8)
4	104	709	64	154	1031 (15.6)
5	49	294	22	40	405 (6.1)
6	208	791	123	122	1244 (18.9)
Totals	936	4431	588	633	6588 (100)

menstrual bleeding (9% spotting and 5% BTB), and 35% reported dysmenorrhea before treatment.

At the time of enrollment into the study, 32% of the patients had already given birth; of these women, 88% had one or two children. Miscarriages and abortions had been experienced by 11% of the patients; of these women, 96% had had one or two such experiences. When questioned about their use of contraceptives, 54% had not used contraceptives previously, and 46% had used oral contraceptives. Triphasic formulations were the most common preparations used. Patients were directly switched over to the new oral contraceptive without a washout period.

Gynecologic or other concomitant illnesses were documented among 4.1% (2473) of the 59,701 patients. The most frequently reported disorders were gynecologic, reported by 1285 patients, and metabolic or endocrine, reported by 718 patients. Infections of the cervix were the most frequently reported gynecologic disorder (17%), followed by those of the breast (8%). Disorders of the thyroid gland were reported by 669 patients. Of the subjects enrolled, 1490 patients received concomitant therapy; thyroid medications were the most frequently used (by 923 patients). The high number of patients taking thyroid medications can be explained by the fact that iodine supplements are required in some parts of Germany and were evidently considered thyroid medication.

Efficacy. Efficacy was evaluated over 342,348 cycles. Pregnancies occurred in 71 women during the treatment period. Thus the use-efficacy Pearl index was 0.25 pregnancies per 100 woman-years; the 95% confidence interval was 0.19 to 0.31.

Tolerability. In all measures of tolerability, only the 42,022 patients who completed six cycles of NGM/EE were included.

Clinical variables. NGM/EE resulted in no significant changes in body weight, blood pressure, or pulse rate after six cycles of treatment. Mean body weight increased 0.6% over initial values. Mean systolic and diastolic blood pressures increased 0.5% and 1.3%, respectively, and mean pulse rate decreased 0.7% over the initial values.

Menstrual cycles. NGM/EE resulted in excellent cycle control, as demonstrated by the low incidence of BTB, intermenstrual spotting, and amenorrhea. The percentage of patients reporting BTB decreased from 4.5% before therapy to 3% after six cycles of NGM/EE treatment. Ninety-four percent of the patients reported no BTB either before the study or during cycle 6 of therapy. In 3% of the patients BTB was no longer present compared with before therapy. Only 2% reported a first episode of BTB, and 1% reported BTB before therapy and in cycle 6.

Similarly, the percentage of patients experiencing intermenstrual spotting decreased from 9% before the study to 4% after six cycles of NGM/EE treatment. Eighty-eight percent of subjects reported no spotting either before entry or during cycle 6 of therapy. Of the remaining 12%, 8% reported discontinuation of spotting after six cycles of NGM/EE, 3% who had not experienced spotting before they entered the study reported it during cycle 6, and 1% reported spotting both before therapy and in cycle 6.

Amenorrhea (no bleeding in a given cycle) occurred in only 1.4% of the women during cycle 6.

The length of cycle 6 was unaffected by NGM/EE in 62% of the patients, whereas it was shortened in 27% and lengthened in 11% compared with baseline values. The duration of bleeding in cycle 6 decreased or remained the same in 91% of patients (53% and 38%, respectively) and increased in only 9% of patients. Also, the severity of bleeding remained unchanged or was ameliorated in most patients in treatment cycle 6. Whereas 96% of patients experienced no change (64%) or a decrease in severity (32%), only 4% reported an increase compared with baseline values.

NGM/EE demonstrated no adverse effect on the reproductive tract during this study. Gynecologic disease was identified in 0.5% of the women at the beginning of the study and in 0.4% at the end of cycle 6.

Laboratory values. Total serum cholesterol, triglyceride, and blood glucose levels were evaluated in 7% of the study population before the study and after the sixth cycle of NGM/EE treatment. Cholesterol and triglyceride levels were assessed enzymatically. The number of patients with serum cholesterol, triglyceride, and blood glucose levels within normal limits at entry remained virtually unchanged during treatment. Of the 2197 patients who entered the study with total choles-

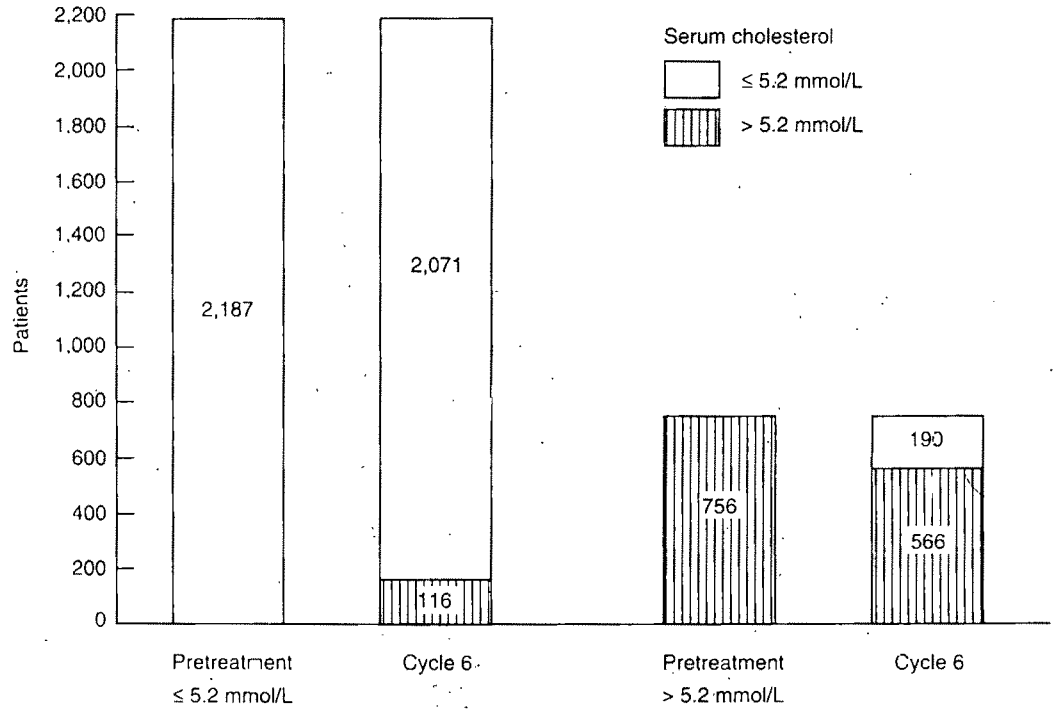


Fig. 1. Numbers of patients with serum cholesterol levels ≤5.2 and >5.2 mmol/L before and after six cycles of treatment.

terol levels in the low or normal range (<5.2 mmol/L), 95% still had low or normal levels after six cycles of NGM/EE, and 5% had increased levels (Fig. 1). Of 756 patients who entered the study with elevated cholesterol levels (≥5.2 mmol/L), 75% had levels remaining above 5.2 mmol/L, and the remaining 25% had normal or low cholesterol levels (<5.2 mmol/L) after six cycles of NGM/EE.

Similar patterns were observed for serum triglyceride levels (Fig. 2). Of the 2075 patients who entered the study with low or normal triglyceride levels (<1.7 mmol/L), 96% still had low or normal levels after six cycles of NGM/EE. On the other hand, of the 500 patients who entered the study with high triglyceride levels (≥1.7 mmol/L), 72% still had high levels, and 28% had normal levels of triglycerides after six cycles of NGM/EE.

Blood glucose levels appeared to be normalized by NGM/EE treatment (Fig. 3). Of the 2738 patients entering the study with low or normal fasting serum glucose levels (<6.4 mmol/L), 93% still had normal levels after six cycles of NGM/EE. Similarly, of 139 patients with initially high blood glucose levels (≥6.4 mmol/L), 48% had normal levels at the end of the study.

Adverse effects. The presence of acne, nausea, headaches, and breast tension (tightness in the breasts) was reported by each patient on her study calendar. The effect of NGM/EE on these side effects was evaluated by comparing the percentage of patients experiencing these symptoms at the pretherapy office visit with the percentage recording them after six cycles of treatment. Information obtained from the personal histories of 41,208 patients indicated that 35,556 (70%) never had acne either at any time before the NGM/EE study or during it. The incidence of acne was reduced from 12% during the pretherapy cycle to 9% after the six cycles of treatment. At the end of the sixth cycle of treatment, 8% of patients described their acne as persisting since before therapy, whereas 5% reported that it was no longer present. Only 1% of the patients who did not report acne before the study experienced it during the sixth cycle.

The incidence of headache also decreased during the study. Headache, which occurred in 13% of women before NGM/EE therapy, was reported by only 5% of subjects after six cycles of treatment. Headaches experienced before the study improved in 9% of women, and only 2% of subjects reported worsening of their headaches during cycle 6 of the study.

Nausea decreased from a pretreatment incidence of 6% to 4% during the sixth cycle of treatment. Although 5% of subjects reported that nausea was no longer present, 1% reported it as unchanged, and 1% experienced nausea for the first time during the sixth cycle.

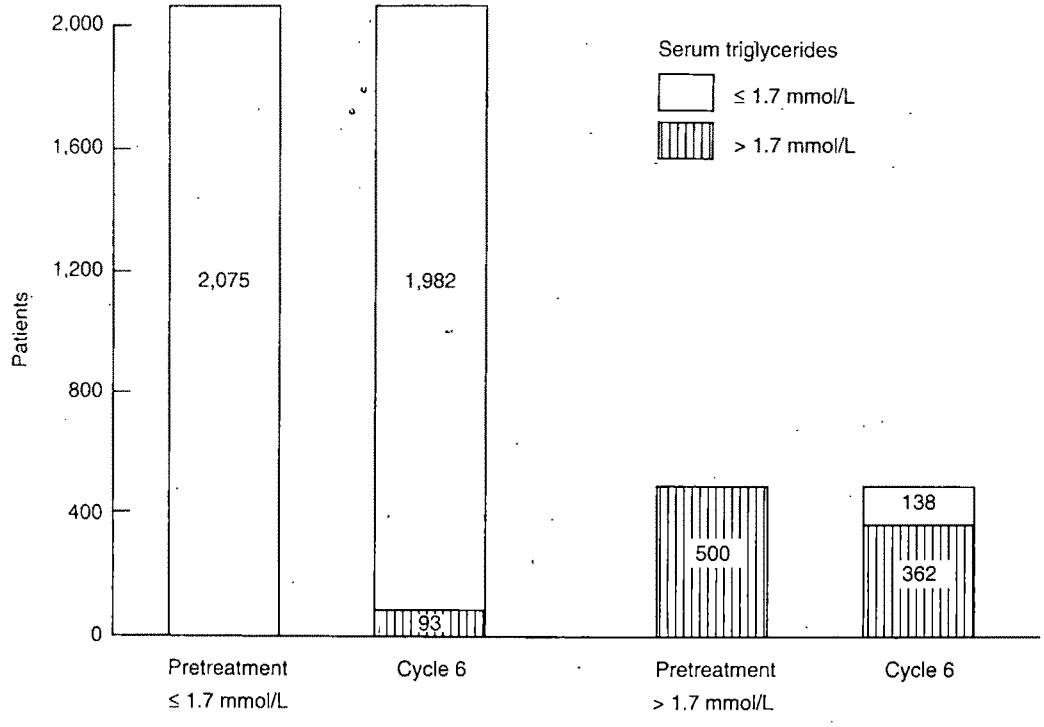


Fig. 2. Number of patients with serum triglyceride levels ≤1.7 and >1.7 mmol/L before and after six cycles of treatment.

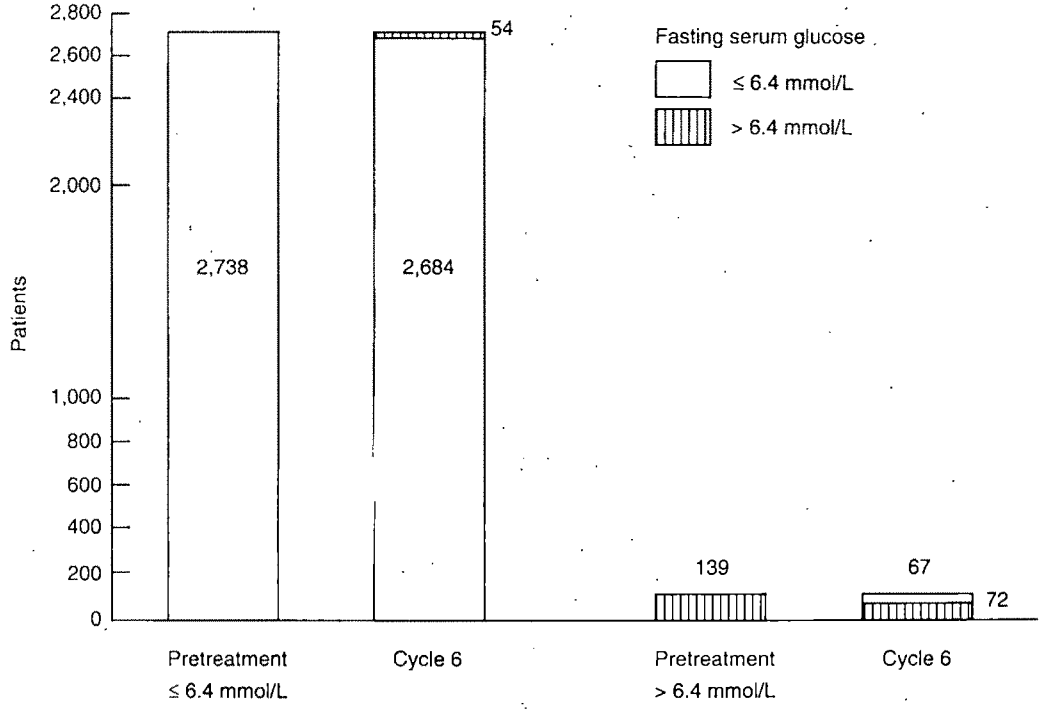


Fig. 3. Number of patients with fasting serum glucose levels ≤6.4 and ≥6.4 mmol/L before and after six cycles of treatment.

Breast tension (tightness in the breasts) was reported by 12% of subjects before they entered the study. At the end of the study, 4% of subjects no longer reported this side effect. However, 9% of patients reported breast tension as a new side effect after six cycles of NGM/EE.

Comment

The results of the present study, in which 59,701 women received NGM/EE for up to six cycles, for a total of 342,438 menstrual cycles, support those of previously reported multicenter trials, as reviewed by Corson[6] and Becker.[9] The use-efficacy Pearl index of 0.25 reported in the current study is similar to the Pearl index of 0 in a 12-month European multicenter open study of 147 women[10] and 0.64 in a 24-month U.S. comparative study of 1473 women (compared with a Pearl index of 0.62 for norgestrel).[6]

The adverse effects observed in the current study are also similar to those noted in earlier trials. In the 12-month open clinical trial (Cilag International Medical Documentation, Medoc-Report NRG 08238, 1989), only 16 adverse effects were reported by 11 patients. In that study NGM/EE had no effect on mean body weight or blood pressure. The mean duration and intensity of menstrual flow decreased during therapy. As expected, BTB and spotting are elevated (3% and 13% of patients, respectively) during the first cycle but returned to baseline levels by cycle 3/4, and amenorrhea never occurred.[10]

Other studies confirm the safety and efficacy of NGM/EE. A small study (Cilag International Medical Documentation, Medoc-Report NRG 05301, 1985) (19 patients) conducted in Germany demonstrated that NGM/EE resulted in no relevant alterations in the coagulation system after the six cycles of treatment. Another study (Cilag International Medical Documentation. Medoc-Report NRG 05288, 1985) (21 subjects; six cycles) demonstrated that NGM/EE had very low androgenic activity, as determined by high levels of sex hormone–binding globulin and low levels of free testosterone. In the same study levels of follicle-stimulating hormone, luteinizing hormone, estradiol, progesterone, and prolactin confirmed the contraceptive efficacy of NGM/EE. Furthermore, no clinically significant changes in fasting blood glucose or basal insulin levels were reported during this six-cycle study. Serum cholesterol and triglyceride levels, which were reduced in cycle 3, returned to baseline levels in cycle 6. Low-density lipoprotein cholesterol levels decreased in both cycles 3 and 6, high-density lipoprotein cholesterol levels increased during NGM/EE treatment.

In the current study, reductions in cycle length and duration of bleeding of 4% and 16%, respectively, were noted; 32% of the women experienced reductions in the intensity of bleeding. Similarly, spotting and BTB decreased over time, with 97% of women reporting an absence of irregular bleeding by the end of cycle 6. Weight, blood pressure, and pulse were minimally affected. The percentage of women who experienced acne, nausea, or headache at the end of six cycles of treatment was lower than the percentage with these symptoms before treatment with NGM/EE.

In general, for patients entering the study with normal levels of total serum cholesterol, serum triglycerides, and blood glucose, these levels were still within the normal range after six cycles of NGM/EE treatment. Of those patients entering the study with high cholesterol and serum triglyceride values, 25% and 28%, respectively, had normal levels after six cycles of NGM/EE treatment. In 50% of patients entering the study with high blood glucose levels, these levels were normal after six cycles of NGM/EE. Thus NGM/EE had minimal effects on total serum cholesterol, serum triglyceride, and blood glucose levels. These data are consistent with the more rigorous evaluations of these metabolic effects obtained in earlier trials.[6, 9]

To date all evidence indicates that NGM/EE is an effective oral contraceptive with a favorable profile for safety and patient tolerance, characterized by neutral or beneficial effects on metabolic functions and a low frequency of minor adverse effects.

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Norgestimate: A clinical overview of a new progestin

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The efficacy and safety of a new monophasic oral contraceptive, norgestimate/ethinyl estradiol, containing the third-generation progestin, norgestimate (250 μg), and ethinyl estradiol (35 μg), are reviewed. Norgestimate/ethinyl estradiol demonstrates excellent contraceptive efficacy, with a Pearl index of 0.25. Cycle control is reliable, with a low incidence of breakthrough bleeding and spotting. Because of the minimal androgenicity of norgestimate, norgestimate/ethinyl estradiol has a low impact on carbohydrate and lipid metabolism. It neither reduces the vasodilatory and antiaggregatory prostacyclin nor increases its endogenous antagonist, thromboxane. Norgestimate/ethinyl estradiol has no significant effect on blood coagulation factors. All these characteristics suggest that norgestimate/ethinyl estradiol may be associated with a lower risk of cardiovascular disease than other oral contraceptives currently available. Epidemiologic data, however, are not available, and physicians should be reluctant to prescribe it or any oral contraceptive to patients who have a history of vascular or thrombotic disorders. (Am J Obstet Gynecol 1992;166:1969-77.)

Key words: Progestins, prostacyclin, cardiovascular disease

Although all oral contraceptives are highly effective in preventing pregnancy, serious questions remain concerning their effect on cardiovascular disease, which is the leading cause of death in women in the United States.[1] Oral contraceptives have been found to induce changes in carbohydrate and lipoprotein metabolism that are similar to those associated with an increased risk of cardiovascular disease, primarily myocardial infarction and stroke. These changes include increases in insulin resistance and low-density lipoprotein (LDL) cholesterol, the most atherogenic of plasma proteins, as well as a decrease in serum high-density lipoprotein (HDL) cholesterol.[2-5] Many investigators have suggested that the negative impact of oral contraceptives on carbohydrate and lipid metabolism plays a major role in increasing the risk of cardiovascular disease by means of atherosclerosis.[6,7] This role is now being challenged, however, because the cause of myocardial infarction among women who use oral contraceptives has been determined to be thrombotic rather than atherosclerotic, and myocardial infarction occurs primarily in women older than 35 years of age who smoke cigarettes.[8-10] Thus a more rational explanation of the increased risk of cardiovascular disease may be that oral contraceptives induce a reduction in vasodilatory and antiaggregatory prostacyclin or an increase in its endogenous antagonist, thromboxane (TXA_2).[11]

Although epidemiologic studies indicate that myocardial infarction is extremely rare in nonsmoking oral contraceptive users, the evidence suggests there is a synergistic effect of contraceptive steroids and smoking on both the risk of myocardial infarction[10] and the inhibition of prostacyclin.[11] This hypothesis does not eliminate the important contribution of lipid alteration, however, because a reduction in HDL decreases prostacyclin synthesis in vitro[12,13] and urinary excretion of the metabolites of prostacyclin directly correlates with serum HDL_2 cholesterol levels.[14] Although it is known that decreased levels of HDL and particularly its subfraction HDL_2 are associated with the risk of coronary heart disease in the general population,[15] this relationship remains to be established in women who use oral contraceptives. However, considering the prevalence of vascular risk factors in women older than 30 years of age (Table I), it is only prudent to strive to maintain a normal blood lipid profile.[16,17]

British,[18-20] Swedish,[21] and U.S.[22,23] studies have suggested that the incidence of venous thromboembolism decreased when the estrogen dose in oral contraceptives was reduced. Although one case-control study[24] was unable to confirm these results, this may have been the result of an insufficient population base.[25] Another explanation for the reduced incidence of thromboembolic disorders in contraceptive users during the past 30 years may be that oral contraceptives have been withheld from women with a history of thromboembolism and in women scheduled for surgery.

Changes in blood coagulation factors have also been studied as a possible explanation for the increased risk of thrombosis and myocardial infarction among women who use oral contraceptives. Oral contraceptives are contraindicated for women with congenital errors of coagulation. Oral contraceptives have been shown to increase various clotting factors (II, VII, VIII, IX, X, and XII) and decrease antithrombin III,[26,27] although

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Table I. Prevalence of cardiovascular risk factors among women aged 30 to 39 years*

Risk factor	*% of women*
Total cholesterol level >6.22 mmol/L	6.4
LDL level >4.14 mmol/L	7.0
HDL level <0.91 mmol/L	5.0
Hypertension	0.5
Obesity (metropolitan relative weight >130 lb)	13.6
Diabetes	0.5
Smokes >10 cigarettes/day	27.2
Parental history of coronary disease before 55 yr of age	32.3
Coronary heart disease	0.5

*Data from Wilson PWF, et al. JAMA 1989;262:41-4.

the magnitude of change in the activity of coagulation factors has not demonstrated any degree of reliability in predicting an individual's risk of thromboembolism. One study[28] suggests that oral contraceptives enhance the formation and resolution of fibrin and leave the hemostatic balance between coagulation and fibrinolysis unaltered. In another study,[29] oral contraceptives enhanced fibrinolytic activity in premenopausal nonsmoking women but not in young smokers.

Carbohydrate metabolism also can be affected by oral contraceptives. There is evidence that androgenic progestins, especially at higher dosages, can cause insulin resistance, increase plasma glucose and insulin levels, and impair glucose tolerance, although these effects rarely result in overt diabetes.[2] Therefore oral contraceptives have been contraindicated for women at risk for diabetes, such as former gestational diabetic patients.

As epidemiologic and clinical data revealed the long-term risks of oral contraceptives, the goal of contraceptive research has been directed toward developing formulations that reduce these risks while retaining optimal contraceptive efficacy and acceptable cycle control. The daily dose of the estrogen component (ethinyl estradiol) has already been decreased to the lowest level that prevents breakthrough bleeding. Now the impact of the progestational component on lipid and carbohydrate metabolism as well as on hemostasis should be minimized to increase the safety of the product. Toward this goal, three new progestins—desogestrel, gestodene, and norgestimate—have been developed for use in oral contraceptives, which are likely to be available in the United States in the early 1990s. These steroids, which represent a new generation of progestins, have lower androgenicity than their predecessors and therefore have different clinical characteristics.[30]

Results of clinical studies of a monophasic oral contraceptive (norgestimate/ethinyl estradiol) that contains norgestimate (250 μg) and ethinyl estradiol (35 μg) demonstrate how one of these progestins has achieved the goal of promoting a more positive lipid profile, has a beneficial influence on prostacyclin metabolism, and has a negligible effect on coagulation factors and carbohydrate metabolism without sacrificing efficacy of patient acceptability. Because of the latency and low incidence of side effects, it will be years before these clinical effects will be reflected in epidemiologic data. However, all indications are that this oral contraceptive may have fewer adverse metabolic effects than those currently available.

Structures of norgestimate and other new progestins

The three new progestins, like those preceding them, are 19-nortestosterone derivatives (Fig. 1). Desogestrel, with its C-11 methylene group, is metabolized to 3-keto desogestrel. Gestodene differs from levonorgestrel in that it has a double bond between C-15 and C-16. Norgestimate has a C-17 acetate group as well as a unique C-3 oxime group that inhibits its ability to bind to androgen receptors in target tissues, thus minimizing the androgenicity of the compound.

Pharmacology of norgestimate

Norgestimate has inherent progestational activity similar to that of progesterone itself (as shown by its strong binding affinity for progestin receptors in the rabbit uterus) (Fig. 2). However, it has minimal androgenicity. This was demonstrated by its relative lack of ability to stimulate the ventral prostate growth in rats, its weak binding affinity (only 0.013 times that of dihydrotestosterone) for rat androgen receptors (Fig. 3), and in its lack of affinity to bind to human sex hormone–binding globulin (SHBG).[31-33] After oral administration to women, norgestimate is well absorbed and extensively metabolized before elimination.[34, 35] Pharmacologic evaluation of the terminal metabolites revealed that 17-deacetyl norgestimate had a pharmacologic profile similar to that of the parent compound. In contrast, two other metabolites, 3-keto-norgestimate and levonorgestrel, were considerably more androgenic than norgestimate in these assays, which makes it unlikely that they made any significant contribution to the biologic activity of norgestimate. Furthermore, 17-deacetyl norgestimate appears rapidly in serum after oral administration. The peak concentrations of this metabolite greatly exceed those of the parent compound, which indicates a substantial conversion of norgestimate to 17-deacetyl norgestimate.[35] In vitro it was identified as the major metabolite of norgestimate in

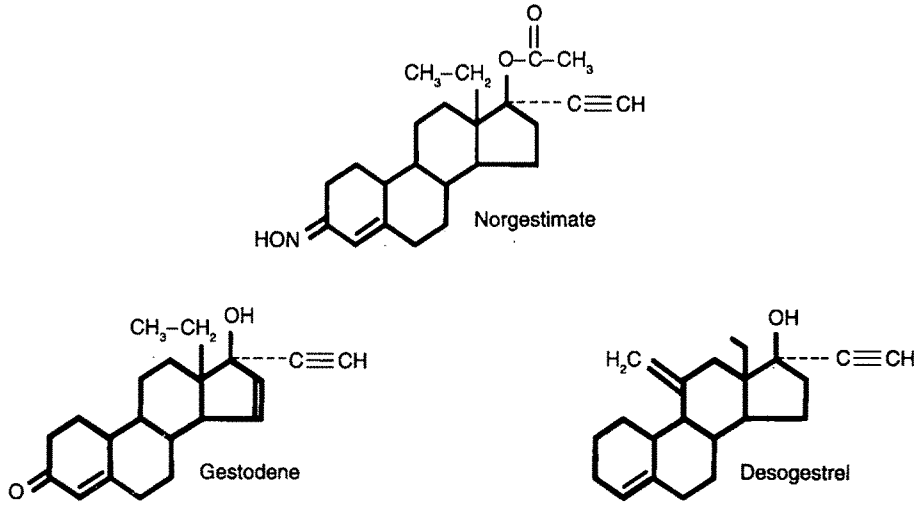


Fig. 1. The new progestins: desogestrel, gestodene, and norgestimate.

human intestinal and hepatic tissues.[36] All these data support the conclusion that 17-deacetyl norgestimate is the major metabolite of norgestimate.

Efficacy of norgestimate/ethinyl estradiol

In a 12-month multicenter German study in which 59,701 women completed 342,348 cycles of treatment, norgestimate/ethinyl estradiol (250 μg of norgestimate/35 μg of ethinyl estradiol) demonstrated excellent contraceptive efficacy, with an overall Pearl index of 0.25.[37] This is consistent with the reported rates of other combination oral contraceptives.[38] Several pharmacologic mechanisms are involved to ensure the compound's contraceptive ability. Evidence that norgestimate/ethinyl estradiol inhibits ovulation was demonstrated by its ability to abolish the preovulatory luteinizing hormone peak (Fig. 4) and to lower plasma estradiol and progesterone to levels incompatible with ovulation.[37] A separate study[39] showed that norgestimate/ethinyl estradiol alters the cervical mucus so that it is impenetrable to sperm. The results of another study[40] demonstrated that the endometrium, which showed neither proliferative nor infiltrative changes during norgestimate/ethinyl estradiol treatment, is most likely incompatible with implantation. These backup mechanisms increase the contraceptive reliability of norgestimate/ethinyl estradiol and more important offer an extra degree of protection against pregnancy in case of a missed pill..

In oral contraception, reliable cycle control is critical to compliance and therefore to the use-effectiveness of the compound. In long-term multicenter comparative clinical trials with a low-dose norgestrel-containing oral contraceptive (0.30 mg of norgestrel plus 0.03 mg of ethinyl estradiol), norgestimate/ethinyl estradiol demonstrated a similar low incidence of breakthrough bleeding and a lower incidence of amenorrheic cycles than did the norgestrel-containing compound. With norgestimate/ethinyl estradiol, clinical side effects, especially those associated with androgenicity, were infrequent, and patient discontinuation because of these side effects was minimal. Weight gain was cited by only 0.84% of subjects who took norgestimate/ethinyl estradiol (vs 1.54% of subjects taking norgestrel/ethinyl estradiol) as the reason for discontinuation, and acne was cited by another 0.84% of patients taking norgestimate/ethinyl estradiol (vs 1.20% of patients taking norgestrel/ethinyl estradiol) as the reason for discontinuation.[41]

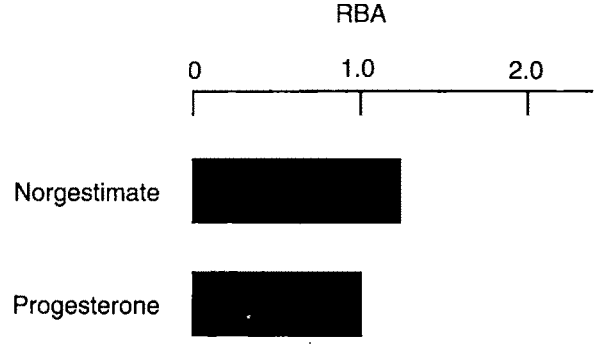


Fig. 2. Relative binding affinities *(RBA)* of norgestimate and progesterone for progestin receptors in the rabbit uterus.

Effect of norgestimate/ethinyl estradiol on SHBG

In addition to binding to androgen receptors and exerting an androgen response directly, a progestin

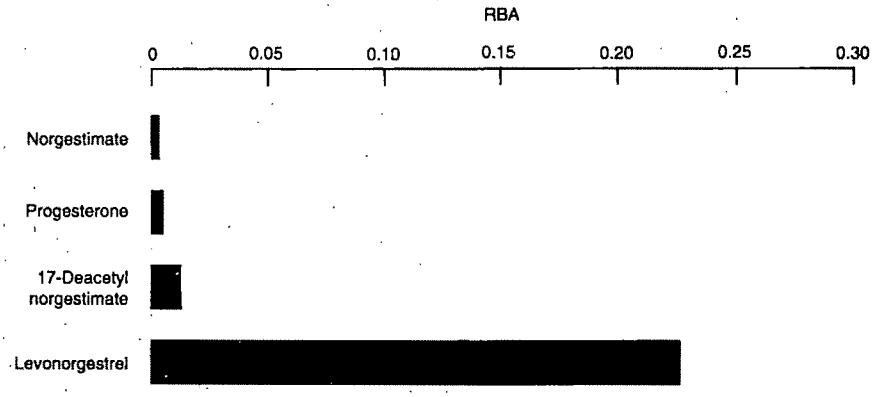


Fig. 3. Relative binding affinities *(RBA)* of norgestimate and its metabolites for androgen receptors in the rat prostate (dihydrotestosterone = 1.0).

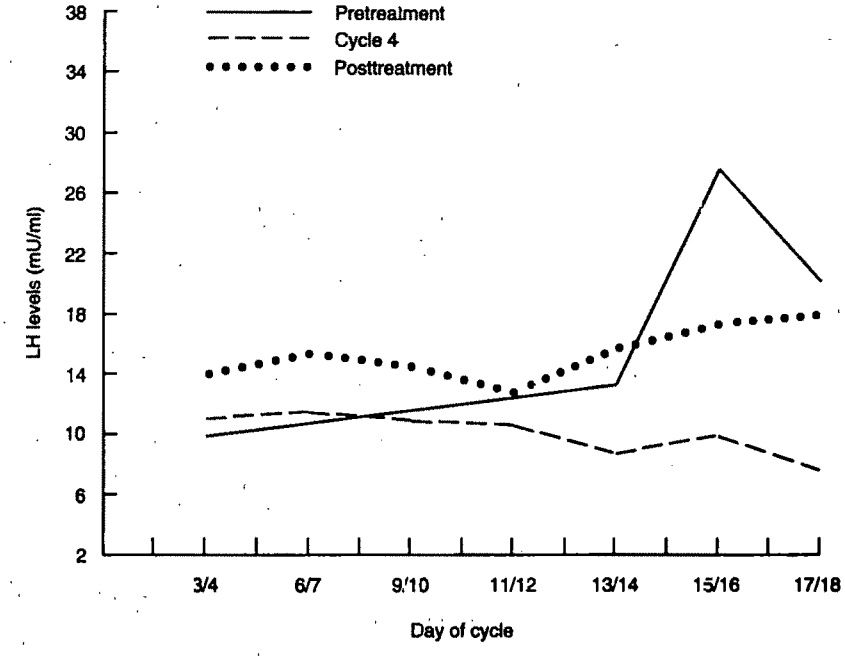


Fig. 4. The effect of norgestimate/ethinyl estradiol on luteinizing hormone *(LH)* levels.

may also cause unwanted androgenic side effects indirectly. Androgenic progestins can do this either by lowering serum levels of the carrier protein, SHBG, or by binding to it, thereby displacing testosterone and resulting in increased levels of free active testosterone (Table II).[42] Estrogens increase SHBG levels, but in women taking oral contraceptives, androgenic progestins tend to oppose the estrogen-induced increase in SHBG.[43] Thus serum SHBG levels can be used to evaluate the balance between the androgenicity of the progestational component and the estrogenic component in women taking oral contraceptives. The results could have clinical implications because decreased serum concentrations of SHBG have been associated with an increased risk of myocardial infarction in postmenopausal women, which strengthens the link between androgenicity and atherosclerosis.[44]

In one clinical study,[45] norgestimate/ethinyl estradiol nearly doubled SHBG levels from their baseline levels after three and six cycles of therapy, while at the same time there was almost a 50% decrease in free testosterone levels. In another study[41] that compared the effects of norgestimate/ethinyl estradiol with those of a norgestrel-containing oral contraceptive, after four cycles serum SHBG levels were between 68.5% and 161.2% above baseline levels in the norgestimate/ethinyl estradiol group compared with only 12.8% to 26.2% above baseline levels in the norgestrel/ethinyl

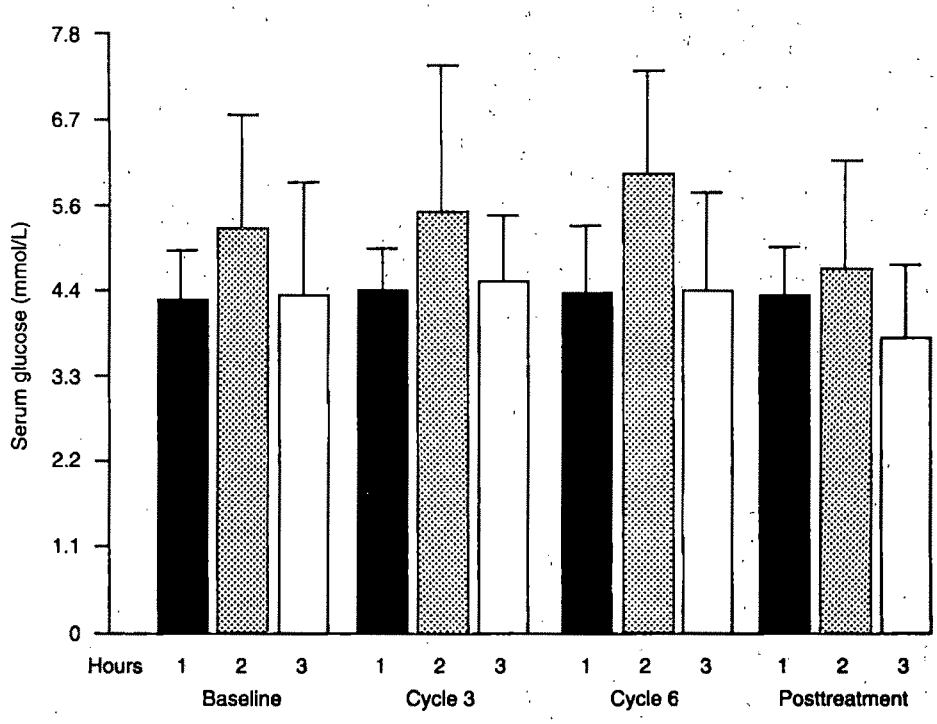


Fig. 5. The effect of norgestimate/ethinyl estradiol on serum glucose at 1, 2, and 3 hours after oral glucose load. There was basically no effect.

estradiol group. These data further confirm the minimal androgenicity of norgestimate.

Glucose tolerance

Most investigators have found that depending on the type and dose of progestin, oral contraceptives have an adverse effect on carbohydrate metabolism and increase blood glucose and plasma insulin.[2, 46-48] The clinical importance of this finding is that impaired glucose tolerance is among the main factors for the development of atherosclerosis,[49] and the impact on the carbohydrate metabolism paralleled the impact on lipid metabolism.[50] Most investigators[30, 51, 52] have found that norethindrone and the new generation progestins, gestodene and desogestrel, have a more favorable impact on carbohydrate metabolism than the more androgenic norgestrel. However, Van der Vange et al.[53] reported that the areas under the curve for both insulin and glucose were unchanged by any of the seven oral contraceptives tested, including levonorgestrel, norethindrone, desogestrel, and gestodene.

The effects of norgestimate/ethinyl estradiol on carbohydrate metabolism were evaluated in 21 women during oral glucose tolerance tests conducted in the third and sixth treatment cycles and again in the second posttreatment cycle.[37] Norgestimate/ethinyl estradiol had no effect on serum glucose levels (Fig. 5). Although insulin levels were slightly higher in the sixth treatment cycle than during pretreatment, they returned to normal range in the second posttreatment cycle. Data on the effects of norgestimate/ethinyl estradiol in gestational, insulin-dependent, and noninsulin-dependent diabetic patients are not available.

Lipoprotein metabolism

Although it is known that high serum levels of LDL cholesterol represent a major risk factor for myocardial infarction and low serum levels of HDL cholesterol are protective against myocardial infarction for women more than 30 years old in the general population,[54, 55] this relationship has not been found to also be true for young oral contraceptive users. However, because HDL increases prostacyclin synthesis in vitro[12, 13] and correlates with urinary excretion or prostacyclin metabolites,[14] it is possible that preventing a decrease in HDL levels could also reduce the thrombotic coronary risk in oral contraceptive users. Whereas the importance of maintaining a normal blood lipid profile with high levels of HDL and its subfraction is well recognized, recent evidence demonstrates that the administration of HDL fractions to rabbits inhibits not only the development and progression of fatty streaks and lipid deposition in the aortic walls in rabbits but can significantly reduce preexisting experimentally induced atherosclerotic lesions.[56]

Norgestimate/ethinyl estradiol results in significant increases in both HDL and HDL_2 levels after six cycles of treatment (Figs. 6 and 7)[37] and significant increases

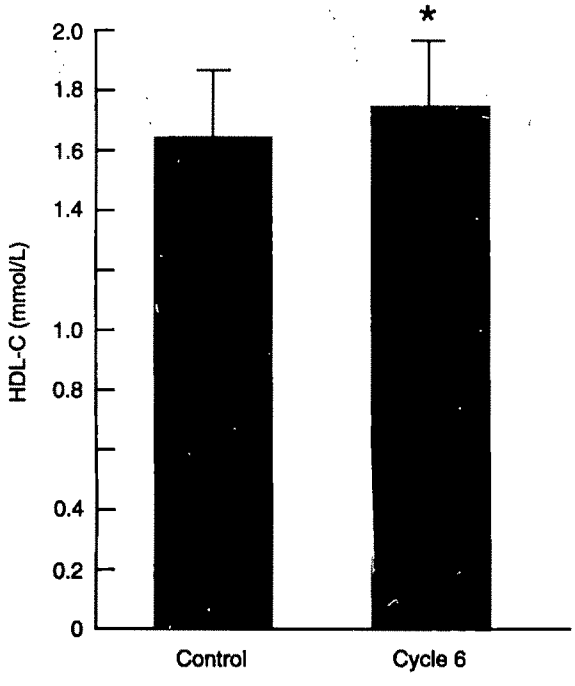


Fig. 6. The effect of norgestimate/ethinyl estradiol on total HDL cholesterol *(HDL-C)*. HDL-C levels increased significantly after six cycles of treatment ($*p < 0.01$).

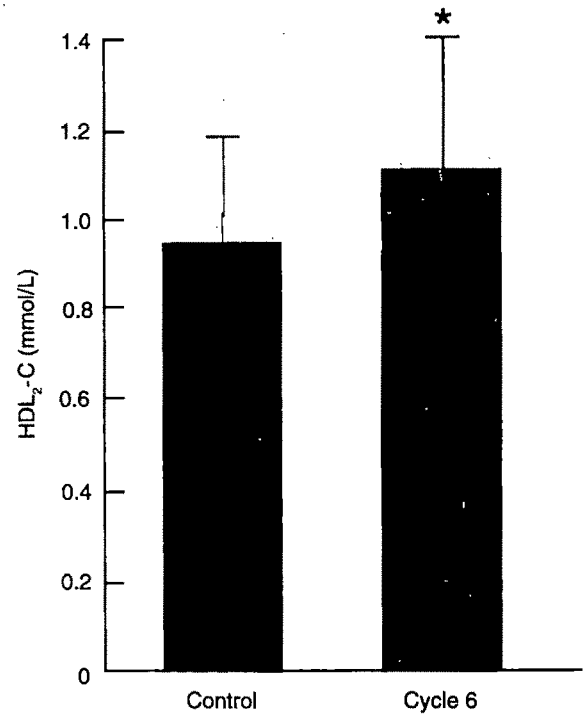


Fig. 7. The influence of norgestimate/ethinyl estradiol on HDL_2 cholesterol *(HDL_2-C)*. There was a significant increase in HDL_2-C levels after six treatment cycles ($*p < 0.01$).

Table II. Characteristics and potential clinical effects of androgenic progestins

Characteristics
↑ Binding to androgenic receptors
↑ Binding to SHBG
↓ Blood SHBG levels
↑ Free testosterone levels
Clinical effects
↓ Serum HDL levels
↑ Serum LDL levels
↓ Serum triglycerides
↑ Insulin resistance
↑ Body weight
↑ Cardiovascular risk

in HDL in treatment cycles 3, 6, 12, and 24 without a significant change in LDL at 12 and 24 months.[41] As expected with the progestins, which have low androgenic activity in combination with estrogen,[57] norgestimate/ethinyl estradiol results in a moderate increase in triglyceride levels.[37] However, data from the Framingham Study suggest that elevated triglyceride levels constitute a cardiovascular risk factor in women only when associated with low HDL levels.[58]

There is a risk in uncritically accepting and interpreting the results of any single study because of the numerous sources of variability in each: age of subjects, smoking, exercise, diet, weight, pretreatment lipid levels, time of day the samples were drawn, and the methodology of analysis of the samples.[59] For example, of 25 published studies of oral contraceptives containing 30 μg of ethinyl estradiol and 150 μg of levonorgestrel, 12 studies reported that the LDL concentration was decreased and 13 reported it was unchanged.[60] However, Chapdelaine et al.[41] have shown that after 24 cycles of norgestimate/ethinyl estradiol (0.25 mg/0.035 mg), mean serum HDL levels were significantly (0.12 mmol/L) above baseline levels, whereas after 24 cycles of norgestrel/ethinyl estradiol (0.30 mg/0.03 mg), HDL levels were significantly (0.07 mmol/L) below baseline levels. These data appear to be reliable, especially in view of the cumulative data showing that norgestrel has significantly more androgenic activity than does norgestimate.

Effects of norgestimate/ethinyl estradiol on prostacyclins

Vasodilatory prostacyclin inhibition or increased vasoconstrictory TXA_2 activity may be important mechanisms for the increased cardiovascular risk among women who take oral contraceptives and smoke cigarettes.[10, 11, 14] There is evidence that smoking decreases prostacyclin formation and increases platelet aggregation in women who use oral contraception.[11]

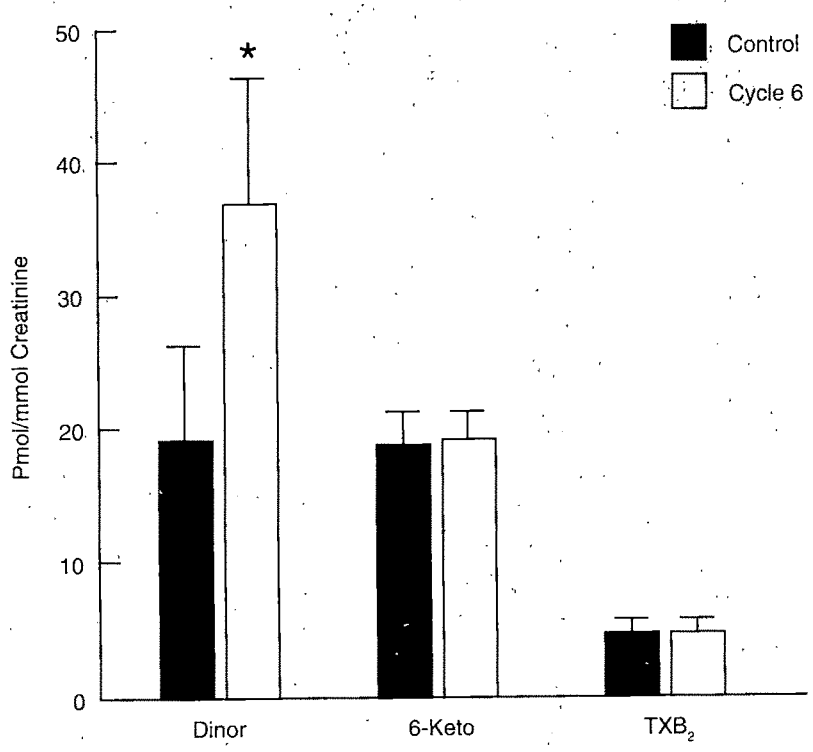


Fig. 8. The effect of NGM/EE on urinary dinor excretion, 6-keto metabolite of prostacyclin, and TXB_2. (*$p < 0.01$).

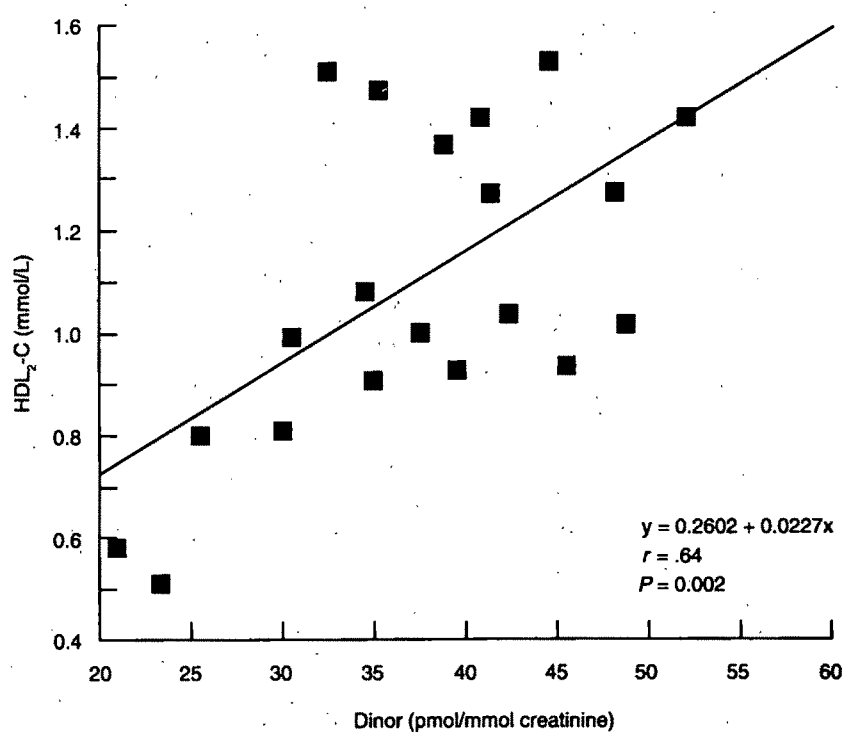


Fig. 9. The relationship of HDL_2 cholesterol *(HDL_2-C)* to urinary excretion of the dinor metabolite of prostacyclin after norgestimate/ethinyl estradiol. (Source of data: Foidart.[61]).

Foidart's study[61] reveals that norgestimate/ethinyl estradiol has no influence on either the 6-keto metabolite of prostacyclin or thromboxane B_2 (TXB_2) metabolite of TXA_2 after six cycles of therapy. On the other hand, urinary dinor excretion, probably the most sensitive index in vivo of prostacyclin, increased significantly (Fig. 8) and correlated with total serum HDL_2 (Fig. 9). These findings suggest that the physiologic regulating system of prostacyclin by HDL is not affected by norgestimate/ethinyl estradiol. Ylikorkala et al.[14] have shown that desogestrel plus ethinyl estradiol has similar beneficial effects: it increased urinary dinor excretion but had no effect on 6-keto prostacyclin or TXB_2 excretion. However, although desogestrel plus ethinyl estradiol increased HDL and HDL_2, there was no correlation between urinary dinor excretion and HDL_2 concentration. Theoretically these increases in prostacyclin metabolites and HDL might decrease the risk of occlusive-thrombotic vascular disorders.

Blood coagulation factors

The association of hypercoagulability with oral contraceptive use is well documented, and these changes are likely to have a role in the risk of thromboembolism and myocardial infarction among women who use oral contraceptives, although long-term epidemiologic studies are needed to show their clinical significance as reliable indicators of risk.[25]

A study conducted in Germany with 19 healthy volunteers demonstrated that norgestimate/ethinyl estradiol produced no changes in fibrinopeptide A (the most sensitive marker of activation of the coagulation system), anticoagulation factors antithrombin III and protein C, or coagulation-promoting factors fibrinogen, factor VII, and the components of factor VIII.[37]

Summary

The effects of norgestimate/ethinyl estradiol on carbohydrate metabolism, lipid metabolism, prostacyclin synthesis, and blood coagulation have been presented in relation to the risk of cardiovascular disease, which is the leading cause of death in women in the United States. Because norgestimate has minimal androgenic activity, norgestimate/ethinyl estradiol has a low impact on these systems. The relative absence of effect of these parameters suggests that norgestimate/ethinyl estradiol may have the advantage of a greater degree of safety over older oral contraceptive formulations. However, long-term epidemiologic studies are needed to determine if the above clinical results will be effective in decreasing the risk of cardiovascular disease associated with older oral contraceptive formulations.

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Discussion I

Chairman Mishell. Dr. Grimes, can you present evidence to support your view that myocardial infarction in women who use oral contraceptives is caused by thrombosis and not atherosclerosis? Will you also discuss why this risk is reduced with moderate- and low-dose oral contraceptive formulations?

Dr. Grimes. The Engel et al. study[1] from Europe probably provides the most compelling evidence. The investigators found that, in the case of women who had been using oral contraceptives and died of myocardial infarction, thrombosis and not atherosclerosis was usually the cause. In addition, evidence shows that women who formerly used oral contraceptives are not at increased risk for myocardial infarction after they have stopped using the oral contraceptives, and no dose-response effect is found.

We do not see this effect with the low-dose pills, as shown in studies by Rosenberg et al.,[2] Stampfer et al.,[3] and others. I believe the evidence is reassuring.

Chairman Mishell. Dr. Grimes, the current trend is to continue to prescribe oral contraceptives for women who are older than 35 years of age. How will this prolonged use of oral contraceptives affect a woman's risk of developing breast cancer in later years?

Dr. Grimes. Again, I believe compelling evidence shows that in general the use of oral contraceptives has no effect on the incidence of breast cancer. However, questions remain about long-term use of oral contraceptives by young women. Only continued studies over the next 15 to 20 years will determine whether the risk of breast cancer is related to the use of low-dose oral contraceptives.

Chairman Mishell. Dr. Grimes, you also showed that high dose oral contraceptives decrease the risk of developing ovarian cysts and endometrial and ovarian cancers. Are the benefits still present with lower-dose agents?

Dr. Grimes. I believe the literature concerning oral contraceptive safety should be rewritten for today's low-dose pills. Two studies that were sufficiently large, however, have looked at low-dose formulations. These are the Cancer and Steroid Hormone Study of ovarian cancer and endometrial cancer.[4,5] These studies reported the same protective effect even with low-dose pills.

Chairman Mishell. How long after menopause should women take oral contraceptives for menopausal symptoms?

Dr. Grimes. In general, my preference is to prescribe hormone replacement therapy, which has a lower dose of steroids than contemporary oral contraceptives.

Chairman Mishell. It is not a lower dose—just a lowered biologic activity—because the steroids are less potent.

Dr. Grimes. Yes.

Chairman Mishell. How long must a woman wait after she stops smoking until she can be considered a safe candidate for oral contraceptive use?

We have data on prostacycline and thromboxane levels; women who smoke and take the pill have a relative excess of thromboxane. After they stop smoking the excess thromboxane disappears, and there is really no evidence of increased cardiovascular risk in women taking oral contraceptives who have stopped smoking. I believe that once a woman stops smoking she is not at increased risk for vascular disease.

Mr. Godsland, we know that estrogen increases the blood supply to the heart and that this effect is partially responsible for the cardioprotective effect of estrogen. Is anything known about the effect of oral contraceptives on coronary artery blood flow?

Mr. Godsland. I know of no evidence regarding the effect of oral contraceptives on coronary artery blood flow. However, to the extent that improvement in coronary artery blood flow appears to be an estrogenic effect, those oral contraceptive combinations that are less progesterone dominant might be expected to have a positive effect on the coronary artery blood flow.

Chairman Mishell. Mr. Godsland, you reported a good effect on high-density lipoprotein and low-density lipoprotein cholesterol with the low-dose estrogen and low-dose progestin pill. Can these new combination pills be used by women who already have hyperlipidemia or hypertriglyceridemia and low high-density lipoprotein levels?

Mr. Godsland. Certainly the newer progestins, in which the effects of estrogen are more apparent, are suitable for a woman who has, for example, a high low-density lipoprotein cholesterol concentration. I think we should be more careful with the more severe lipid abnormalities, such as hypertriglyceridemia. The physician should proceed with caution when the patient has a severe abnormality and should perform serial measurements of lipoproteins during oral contraceptive use. However, if a woman is moderately overweight or has low high-density lipoprotein cholesterol levels, I would be fairly confident about prescribing the new combination pills.

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Chairman Mishell. Do you believe that being overweight by itself is a contraindication for oral contraceptive use?

Mr. Godsland. I would make a distinction between being moderately overweight—between 120% and 135% ideal body weight—and severe obesity. Although I believe that severely obese patients should be carefully monitored, I am not sure an absolute contraindication exists. We need more information.

Chairman Mishell. Dr. Runnebaum, is it safe to prescribe a product with norgestimate for women who have lipid abnormalities?

Dr. Runnebaum. The information we presented in our paper were cumulative data about changes in lipid levels in women who receive the low-dose norgestimate oral contraceptive. We ae now performing further analyses of the pretreatment incidence of different types of lipid disorders, such as hypercholesterolemia, and are assessing the individual lipid changes during oral contraceptive use.

Chairman Mishell. Dr. Bringer, what do you think?

Dr. Bringer. In my opinion the epidemiologic data are insufficient to answer this question. We must be careful when we make individualized decisions and recommendations, because even with the new compound, we have no data at this time on risk factors.

Chairman Mishell. Can a woman undergo elective surgery if she is taking oral contraceptives? If the answer is no, how many days before surgery is it necessary to withdraw the oral contraceptives?

Dr. Grimes. The current recommendation that women discontinue taking oral contraceptives about 30 days before elective major surgery stems from case-control studies performed with older, high-dose pills in the early 1970s in the United Kingdom and the United States. In those studies a three- to sixfold increase in the risk of postoperative thrombosis was seen if a woman had been taking the pill.

In general physicians have not recommended that patients discontinue oral contraceptive use before minor procedures, such as laparoscopy. To what extent those data are relevant to today's low-dose pills remains to be seen.

Chairman Mishell. Dr. Grimes, which factor do you believe has contributed the most to reducing the side effects of oral contraceptives, particularly ischemic heart disease and strokes: the reduced dosages of steroids, the better selection of users (in other words, excluding smokers)?

Dr. Grimes. I am not certain that important question has an answer. We cannot disentangle which factor caused the decline.

Chairman Mishell. Why are products that contain the new progestins not marketed in the United States when they have been marketed in Europe for at least 5 years?

Dr. Grimes. That is an imponderable question. We must deal with regulatory agencies in the United States.

Chairman Mishell. I believe that these products will be marketed in the United States within the next few months. At least, we are hopeful they will be available because of their demonstrated value, as shown in the studies presented at this symposium.

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Introduction to steroids in the menopause

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Menopausal symptoms and signs associated with the reduction of ovarian function include an increased incidence of cardiovascular disease and loss of bone mass as well as less serious but more uncomfortable symptoms such as vasomotor flushes and atrophy of the vaginal wall. Although unopposed estrogen effectively reverses these and other menopausal symptoms, it is well established that without the addition of progestin there is an unacceptably high risk of developing hyperplasia or cancer of the endometrium. Depending on the type and dose of progestin added, however, this addition may reverse estrogen's beneficial cardiovascular effects and produce unwanted side effects. Lower doses and newer progestins, such as norgestimate, gestodene, and desogestrel, have demonstrated a decreased potential to reverse the positive cardiovascular effects of estrogen while still eradicating persisting or de novo endometrial hyperplasia. (AM J OBSTET GYNECOL 1992;166:1980-5.)

Key words: Progestin, hormone replacement therapy, menopause

In addition to mitigating vasomotor symptoms and alleviating urogenitary discomforts, estrogen replacement therapy favorably influences the incidence of fractures and cardiovascular disease. The symptomatic relief of estrogen deficiency symptoms has long been recognized. Despite the fact that 80% of women at some time experience symptoms of estrogen deficiency, only a few receive replacement therapy. Because of various, mainly nonmedical factors, the percentage of women in industrialized countries who use hormone replacement therapy varies from 4% to 40%. The duration of therapy is usually short (6 months to 3 years) in most countries.

Including preventive measures as an indication for hormone replacement therapy should lead to extended treatment periods provided that safety is maintained. Available cost-benefit analyses agree with the assumption that lives could be saved if hormone replacement therapy was used more extensively. In addition, results from several studies[1, 2] show that quality of life is greatly improved in women using hormone replacement therapy compared with age-matched control subjects. Hence a growing body of evidence suggests that hormone replacement therapy increases not only quality but also quantity of lives. Yet hormone replacement therapy remains little used. Excluding the positive cost-benefit analyses, several other factors may lead to a rapid increase in the use of hormone replacement therapy within the next few years.

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6/0/37615

Factors leading to increased use of hormone replacement therapy

The number of women reaching the climacteric is rapidly growing as those women born after World War II now approach menopause. Generally this generation is better educated, partly as a result of input from the mass media such as television and radio. With the introduction of oral contraceptives in the early 1960s, it was suddenly possible to avoid an undesired pregnancy. With this accomplishment, it is inconceivable that this generation would accept the difficulty of menopausal symptoms. Furthermore, many of these women already have personal experience with hormonal medication such as oral contraceptives.

In Europe some women of climacteric age complain about the noninterest and unwillingness of physicians, including some gynecologists, to recommend hormone replacement therapy.[3] A recent survey among general practitioners in Norway also confirmed that younger physicians and female colleagues were more prone than older and male colleagues to discuss hormone replacement therapy with women of climacteric age. There was also a correlation between interest in hormone re-

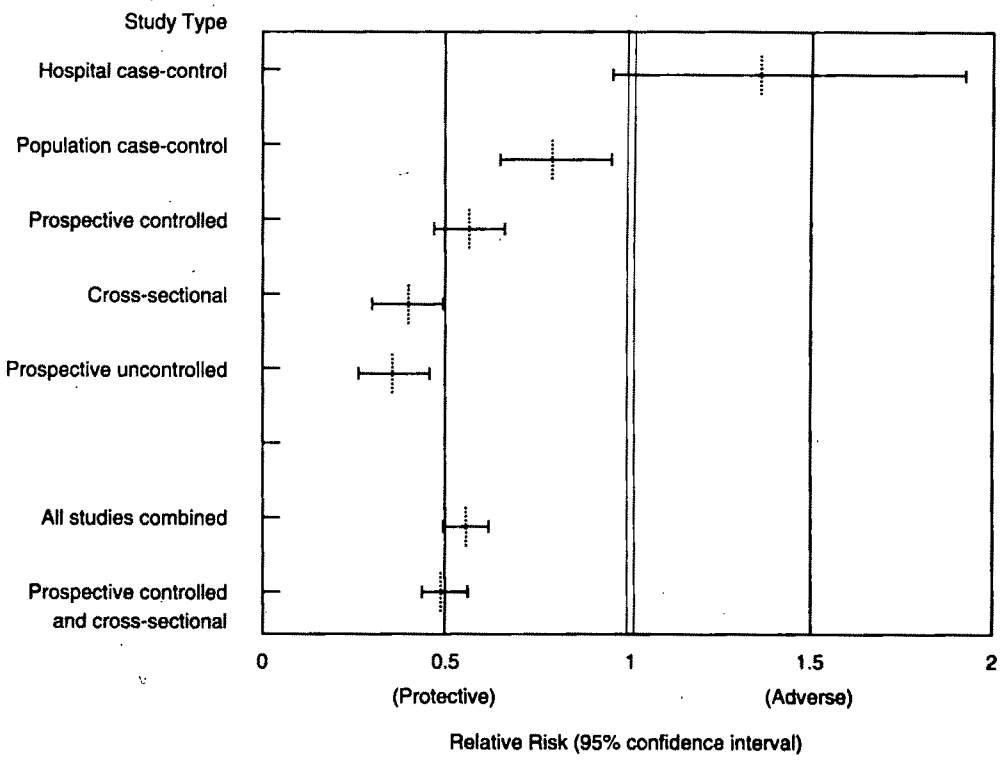


Fig. 1. Comparison of relative risks of coronary heart disease by study type among women receiving estrogen therapy. (From Stampfer MJ, Colditz GA. Prev Med 1991;20:47-63.)

placement therapy and the physician's knowledge about the pros and cons of such regimens.

Information seems to be the primary need not only to gynecologists but also to journalists and politicians as well as to women and men in general. Such information should in particular be directed to cardiologists, orthopedic surgeons, geriatricians, and general practitioners, who are consulted by a considerable part of the female population of menopausal ages. It is also important to address the issue of potential health benefits to those women who do not experience vasomotor symptoms. A more detailed outline is difficult, because it would depend on the medical infrastructure of each community.

Treatment periods may also be lengthened. The evidence suggests that estrogens and estrogen-progestin combinations prevent osteoporosis. Estrogens are mainly considered antiresorptive agents, but recent reports[4, 5] imply that estrogens could be used for the treatment of established osteoporosis, a fact that may be of great importance for future health strategies. Several consensus committees have concluded that estrogen is most effective for preventing osteoporosis during the years surrounding menopause, which in turn would lead to treatment periods of 10 to 15 years.

The cardioprotective effect of estrogens has also been documented (Fig. 1). Admittedly, results from large, multicenter, controlled clinical trials are unavailable, but observational data are impressive and consistent with the notion that estrogens reduce cardiovascular disease by 50%.[6] Most studies of estrogen replacement therapy and cardiovascular disease enroll women with no previous manifestations of arterial disease. The arteriosclerotic plaque serves as an outstanding matrix for the formation of a clot. It would therefore seem possible that estrogen use could be potentially hazardous for women with advanced arteriosclerosis, provided that estrogens promote thrombotic events. In the Leisure World Study, Henderson et al., and Paganini-Hill et al.[8] demonstrated that women with previous myocardial infarction or stroke also benefitted substantially from estrogen therapy. In fact the protective effect seemed to be the same regardless of previous history of arterial disease. Looking more specifically at this problem, Bush[9] in the Lipid Research Clinics study found that the relative risk of subsequent arterial disease in women with a past history of myocardial infarction was 0.2. Thus it seems as if those subjects who were at the highest risk had the most to gain from estrogen replacement therapy. Data from Sullivan et al.[10] comply with this notion, as angiographically documented arteriosclerosis diminished during estrogen

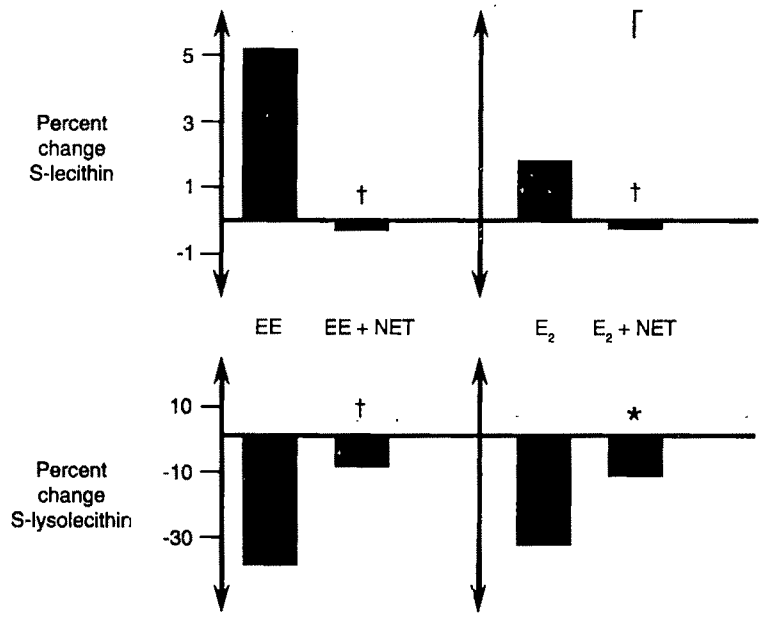


Fig. 2. Changes in lecithin and lysolecithin content of LDL during estrogen replacement therapy. *EE*, Ethinyl estradiol; *NET*, norethindrone; E_2, estradiol. $*p < 0.01$; $\dagger p < 0.001$. (Modified from Silfverstolpe G, et al. Acta Endocrinol 1981;95:527-33.)

intake. The most dramatic reduction was seen in advanced cases. If these data can be confirmed, estrogen therapy would be one of the more efficacious secondary prevention measures for women with previous myocardial infarction.

Current users of estrogen replacement therapy seem to have a far better "protection" from cardiovascular disease than do former users, who in turn are better off than nonusers.[11] These observations may imply that besides influencing atherogenesis, other mechanisms that improve cardiovascular function are also exerted by estrogens. Such mechanisms include vasodilatation and reduced vascular resistance, which in turn lead to an increase in blood flow.[12, 13]

Also of interest is the observation that lowering low-density lipoprotein (LDL) also improves rheologic properties of blood.[14] The clinical relevance of such changes are supported by the findings of enhanced blood flow to skeletal muscle and higher muscle tension of oxygen after extracorporal removal of LDL (LDL-apheresis) in patients with hypercholesterolemia.[15] Furthermore, experimental arteriosclerosis induced by high cholesterol levels is accompanied by an increase in vascular tone.[15] These findings may be related to impaired function of the endothelium-derived relaxing factor. Whether synthesis, release, or breakdown of endothelium-derived relaxing factor is affected by LDL cholesterol remains to be clarified.[16] Recent data[14] also suggest that hemodynamic changes occur before the gross appearance of an atheroma. The changes in cholesterol metabolism and the concomitant reduction in blood pressure and improvement in hemorrheology in women who use estrogen remain to be clarified.

It should be noted, however, that measuring quantitative lipid changes may not be the full answer to the lipid-arteriosclerosis relationship. It has recently been established that qualitative changes, especially in LDL, play an important role in atherogenesis. Even minimally modified LDL particles may dramatically increase their atherogenicity. Such modifications in LDL comprise above all oxidative processes. In addition to it being highly atherogenic, oxidized LDL is characterized by a low content of polyunsaturated fatty acids and lecithin and a higher content of lysolecithin in comparison with native LDL. Estrogens increase lecithin and polyunsaturated fatty acids in plasma, concomitant with a reduction of lysolecithin (Fig. 2).[17] It would seem likely that the multifactorial effects of estrogen in reducing cardiovascular disease also include the reduced formation of modified (oxidized) LDL particles.

Furthermore, dietary supplementation of n-3 fatty acids in pigs has resulted in augmented release of endothelium-derived relaxing factor from coronary artery segments as well as increased sensitivity of vascular smooth muscles to the effects of endothelium-derived relaxing factor.[18] Because estrogen-progestin combinations may influence the relative fatty acid composition,[19] other intriguing possibilities for estrogen effects may arise.

The plasma concentration of estradiol after the administration of nonoral estrogens, such as those used in the transdermal patch, is similar to that of the fol-

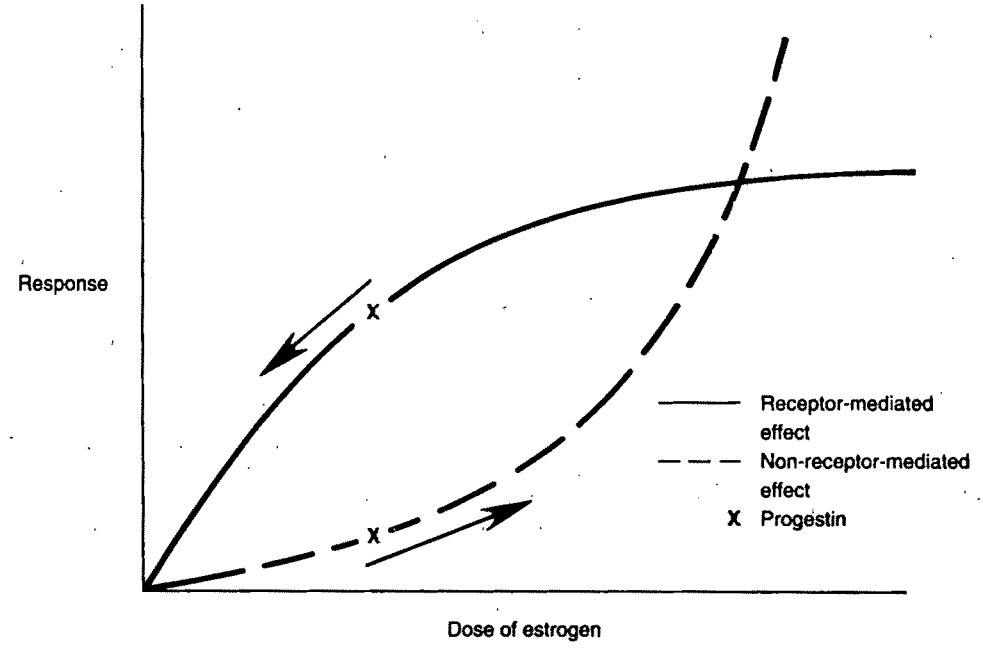


Fig. 3. Variable dose responses to estrogen and progestin.

licular phase and hence more closely resembles physiologic conditions than does oral therapy. A growing body of evidence suggests that most of the cardioprotection offered by estrogens is accomplished by direct effects on the vascular wall that comprise both biochemical (e.g., effects on LDL oxidation and endothelium-derived relaxing factor) and physiologic events. Therefore nonoral estrogen delivery systems are likely to be as beneficial as oral estrogens in relation to cardioprotection even though the plasma biochemical profile might be different. By avoiding the first passage through the liver, nonoral estrogens display less marked changes in liver-derived constituents as exemplified by factors belonging to the coagulation cascade. In addition, transdermal patches seem to lower triglyceride and LDL-cholesterol levels, thereby leaving high-density lipoprotein (HDL) levels virtually unchanged.[20, 21]

Use of progestins in estrogen replacement therapy

Whether progestins per se influence the incidence of cardiovascular disease is unknown. There is no natural situation in which progestogen is secreted without the concomitant secretion of estrogens. Our current understanding is based on reports in women who use oral contraceptives or are undergoing treatment for endometriosis or malignancies of the endometrium or breast, and results are blurred by the underlying disease. In fertile women the overall impression is that progestin administration does not increase the risk of cardiovascular disease more than depletion of ovarian function would suggest.

A decade ago data appeared in the literature indicating a metabolic difference between 17α-hydroxyprogesterone derivatives and progestogenic compounds derived from 19-nortestosterone. These data were based on studies comprising only three progestins, namely, medroxyprogesterone acetate as a member of the 17 α-hydroxyprogesterones and levonorgestrel and norethindrone as examples of 19-nortestosterones. However, more recent studies[22, 23] on other progestogenic compounds have not verified the extrapolation of the data on these three progestins to be valid for all progestins. Marked metabolic differences between progestins exist, but the reasons are more complicated than a mere dissimilarity in parent chemical structure. Earlier preparations of medroxyprogesterone acetate had a poor oral bioavailability,[24] which to some extent explains its relative inertness in previous work. By improving its absorption, medroxyprogesterone acetate now shows some of the characteristics of other progestins, for example, lowering of HDL levels.[25, 26]

In several physiologic and biochemical events, progestins counteract the effects of estrogens on insulin concentrations, triglyceride levels, and total and HDL- and LDL-cholesterol. They also produce vasoconstriction of estrogen-primed arteries. Therefore it is natural to assume that the addition of progestin might harm estrogen-induced cardioprotection. However, the scare epidemiology that exists does not support such an assumption. To date only three studies[27-29] have addressed

the problem of cardiovascular disease and combined therapy. However, all three suggest that the progestin does not negatively influence cardioprotection. The progestins involved were norethindrone,[27] medroxyprogesterone acetate,[28] and levonorgestrel.[29] These findings are further supported by experimental data[17] and animal models.[30,31] It should be stressed that in this context pharmacologically induced changes of HDL may not have the same clinical significance in relation to the risk of cardiovascular disease as HDL has in population-based samples.[32]

Although insufficient, the results of these studies render it necessary to have a revised hypothesis regarding the mechanisms by which female sex steroids influence arterial function and integrity. As seems to be the case with bone mineral metabolism, progestins apparently do not counteract the estrogen effects but could in fact show additional or even synergistic effects. A novel hypothesis on mechanisms of action by estrogens and progestins takes into account not only the classic receptor-mediated effects but also effects exerted by nongenomic (i.e., non-receptor-mediated) actions (Fig. 3).[26]

Estrogens and adverse side effects

Safety is of primary concern when dealing with preventive measures. This is further accentuated when the number of users is growing and particularly when treatment periods expand. Estrogens are known to increase the risk of endometrial cancers, a side effect that can be controlled for by adding progestin to the estrogen replacement therapy. Two major problems remain: the breast cancer issue, which will be covered elsewhere, and the bleeding problem. The longer the treatment period, the more distress a patient experiences because of vaginal bleeding, especially if the bleeding is heavy and irregular. The bleeding pattern improves if compliance can be maintained, a fact that has been underlined by the popularity of the fixed estrogen-progestin combination tablets that are available in Europe.

The introduction of continuous combined preparations offers a possibility for a number of women to receive hormone replacement therapy without bleeding and still be protected from endometrial hyperplasia and possibly endometrial cancer. With a growing understanding of the reasons for bleeding from an atrophic endometrium, continuous combined regimens could be improved.[33-35] The introduction of tibolone as treatment for climacteric symptoms is also noteworthy. This steroid combines estrogenic, progestogenic, and androgenic properties in one molecule. Its clinical performance and bone-preserving abilities are good.[36] Data on cardiovascular disease are lacking, but treated patients have lower triglyceride levels than do control subjects and no significant alterations in LDL or HDL.[38]

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The benefits and risks of hormone replacement therapy: An epidemiologic overview

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In postmenopausal women estrogens alone are effective in reversing vasomotor symptoms and vaginal atrophy. They also prevent the bone loss associated with osteoporosis and reduce the risk of cardiovascular disease, probably through their beneficial effects on lipid metabolism. Unopposed long-term estrogen therapy, however, increases the risk of developing endometrial hyperplasia, endometrial cancer, and possibly breast cancer as well. The risk of developing endometrial cancer can be reduced by combining a progestin with the estrogen, by controlling obesity, and by rigorous clinical screening and surveillance. The effect of progestins on the risk of developing breast cancer is still controversial. Although some progestins may reverse the cardioprotective effect of estrogens, those with minimal androgenicity appear less likely to do so. Hormone replacement therapy that combines estrogen with a progestin of minimal androgenicity is thus a rational alternative to unopposed estrogen therapy. Current epidemiologic knowledge suggests that the benefits of hormone replacement therapy, with or without any progestins, strongly outweigh the risks. (AM J OBSTET GYNECOL 1992;166:1986-92.)

Key words: Hormone replacement therapy, estrogens, progestins, cardiovascular disease, breast cancer, endometrial cancer

It has been known for many years that lack of estrogen is the cause of menopausal symptoms. In 1932 Geist and Spielman[1] first proposed that women be given estrogens to reverse these symptoms. In the 1940s a link was established between estrogen deficiency and osteoporosis,[2] and in the 1950s it was recognized that estrogen inhibited atherosclerosis.[3] A clinical trial[4] with men as subjects was performed to test the hypothesis that high doses of conjugated estrogens would prevent coronary heart disease; the trial was stopped when the men who had been treated with estrogen experienced more nonfatal myocardial infarction, pulmonary embolism, and thrombophlebitis than did the untreated men. The trial did not study the more physiologic low doses of estrogens that are now used in postmenopausal women, and women were not studied. Scientists hesitated many years before they accepted that the side benefits of estrogen therapy in postmenopausal women included the long-term prevention of cardiovascular disease.

The short-term benefits of estrogen were recognized early, however, and in the 1960s and 1970s estrogen replacement therapy became increasingly popular. Between 1966 and 1975 the number of prescriptions for estrogen that were dispensed annually doubled.[5] When it became clear that unopposed estrogen replacement therapy was associated with an increased risk of endometrial hyperplasia and cancer,[6,7] however, sales of estrogen dropped abruptly.[5] During the late 1970s the pathogenesis of endometrial hyperplasia was elucidated, and the ability of progestins to antagonize it was discovered. By 1981 the addition of progestins to estrogen therapy had been shown to reduce the incidence of endometrial abnormalities.[8] The use of hormone replacement therapy by postmenopausal women has increased steadily since then.

Many studies have evaluated the long-term safety of estrogen replacement therapy. Most dealt with "unopposed" estrogens because they were used before the introduction of progestin-estrogen combinations. Experience with younger women (i.e., those who use oral contraceptives) has shown that progestins have a range of side benefits and risks that differ markedly from those of estrogens. The new popularity of combined hormone therapy in perimenopausal and postmenopausal women has raised new questions about the overall benefits and risks of such treatment.

Benefits of estrogens

Reduced risk of cardiovascular disease. Overwhelming evidence has been provided by epidemiologic studies of a protective effect of estrogens against cardiovascular disease in women.[9,10] This is true whether the endpoint is myocardial infarction, stroke, or all cardiovascular disease and whether the researchers have studied nonfatal or fatal events or both. Although the results of different studies have varied, the relative risks of cardiovascular disease observed in women who use estrogens have been between 0.2 and 0.7 in prospective

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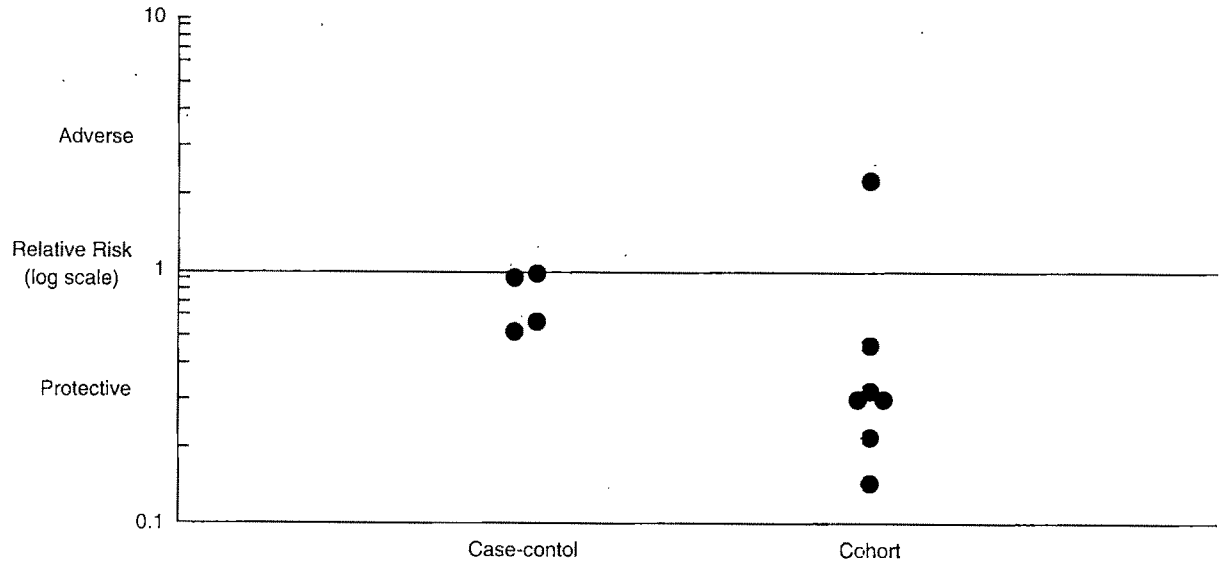


Fig. 1. Relative risks of cardiovascular disease and myocardial infarction in women who use estrogens in retrospective (case-control) and prospective (cohort) studies. Excludes small studies and those based on deaths only. (Data from Ernster et al. Prev Med 1988;17:201-23.)

studies and somewhat higher in retrospective studies (Fig. 1). Studies based only on fatal events have shown similar results.[11] Important cohort studies that have led to this knowledge include the Nurses' Health Study,[11] which followed more than 48,000 women for nearly 338,000 person-years, and found that relative risks in those who used estrogen included 0.56 for major coronary disease, 0.97 for stroke, and 0.72 for total deaths. Another prospective study[12] that followed more than 24,000 women found a relative risk for myocardial infarction of 0.7. Other notable studies include that of the Royal College of General Practitioners[13] (relative risk of myocardial infarction, 0.8); the Leisure World study[14] (relative risk of death from myocardial infarction, 0.47); the Walnut Creek study[15] (relative risk of death from cardiovascular disease, 0.6); the Lipid Research Clinic study[16] (relative risk of death from coronary disease, 0.75 after adjustment for age or 0.99 after adjustment for other risk factors); and the Framingham heart study,[17, 18] in which the relative risks have varied from 0.4 to 2.2, depending on the endpoint, age group, and other risk factors.

Stampfer et al.[12] have recently conducted a meta-analysis of these and other studies of women who use estrogen and showed a summary relative risk of cardiovascular disease in appropriately designed cohort studies of 0.58, whereas other cohort studies together showed higher relative risks. Case-control studies showed the highest relative risks. The authors demonstrated that whereas case-control studies that used population-based controls showed a significantly reduced summary relative risk of 0.76, those that used hospital-based controls showed no such protection. Stampfer et al.[12] and others have argued that this inconsistency is explained by the inadvertent inclusion in hospital-based case-control studies of controls who have conditions that are negatively associated with estrogen use (e.g., fractures).

The protective effect of estrogens against cardiovascular disease does not appear to be explained by the better health of estrogen users, although this point remains controversial.[12] A protective effect has been observed in women of different ages, including older women,[12, 16] smokers, and women who have had an oophorectomy.[12] The protective effect is not clearly related to estrogen dose or duration of use.[12]

Estrogens improve the lipid profile in the direction that is consistent with their beneficial effect on cardiovascular disease. Estrogens increase high-density lipoprotein cholesterol and reduce low-density lipoprotein cholesterol and total cholesterol levels,[19-23] effects that change the risk of cardiovascular disease.[24-27] These effects are seen in women of different ages, in women with and without ovaries, in healthy women, and also in those who have a history of cardiovascular disease. Thus a major proportion of the beneficial action of estrogens is assumed to be through their effects on lipids and subsequently atherosclerosis, although they may also have other effects.

The main cause of death among women (and men) in the United States is cardiovascular disease.[28] Therefore if estrogen replacement therapy resulted in even a small decrease in the relative risk of cardiovascular disease, it would lengthen the life expectancy of a large number of women.

Henderson et al.[29] estimated the beneficial effects of



Fig. 2. Relative risks of cancer of endometrium and breast in women who use estrogen.

estrogens in the number of deaths prevented. At age 65 to 74 years women in the United States would experience approximately 284 per 100,000 fewer deaths annually from coronary artery disease if they currently used estrogen compared with those who do not use estrogen. They also estimated that 49 per 100,000 fewer annual deaths would occur from cerebrovascular accidents, although the relationship with this endpoint to estrogen replacement therapy is now more controversial.[11] One can speculate that the benefit of estrogen replacement therapy might be even greater in smokers, who have a substantially increased risk of cardiovascular disease and are also in greater need of estrogen replacement therapy to prevent osteoporosis.[10]

Reduced risk of osteoporosis and bone fracture. Osteoporosis is a major health problem in elderly women. From 25% to 44% of women experience spontaneous fractures after menopause.[2, 30] Epidemiologic evidence clearly demonstrates that estrogen can retard the development of osteoporosis and substantially decrease the risk of osteoporotic and hip fractures.[11] A minimum of 6 years of estrogen replacement therapy reduces the risk of hip fracture and Colles' fracture by 50%. This protection diminishes after the use of estrogen is stopped. Estrogen protects against fractures of the hip, forearm, and vertebrae.

Henderson et al.[29] estimated that estrogen replacement therapy would reduce the annual mortality rate caused by hip fractures in women in the United States aged 65 to 74 years from 55 per 100,000 to 22 per 100,000.

Long-term risks of estrogen replacement therapy

Increased risk of endometrial cancer. Endometrial cancer ranks third among the most common cancers in women in the United States, with an estimated annual incidence in the range of 39,000 to 42,000 cases.[29, 31] In 1987 alone the National Cancer Institute estimated that 2900 deaths resulted from endometrial cancer and 35,000 new cases of endometrial cancer developed in the United States.[32] Because vaginal bleeding is an early symptom, diagnosis is usually prompt and the prognosis is favorable: the 5-year relative survival rate is 93% among white women with stage I disease and 84% for all stages combined. With or without estrogen treatment, the risk of endometrial carcinoma is known to increase with nulliparity or low parity, obesity, diabetes, and a family history of the disease. It is also recognized that the average age-adjusted incidence rate is significantly higher in white women (21.3/100,000) than in black women (13.1/100,000).[32]

An association between the increasing incidence of endometrial cancer and the increased use of estrogens was made in 1975, when two independent case-control studies[6, 7] reported that the relative risk of endometrial cancer was four to seven times greater among women using estrogen. Since then numerous studies[9] have confirmed this association. Women who use unopposed

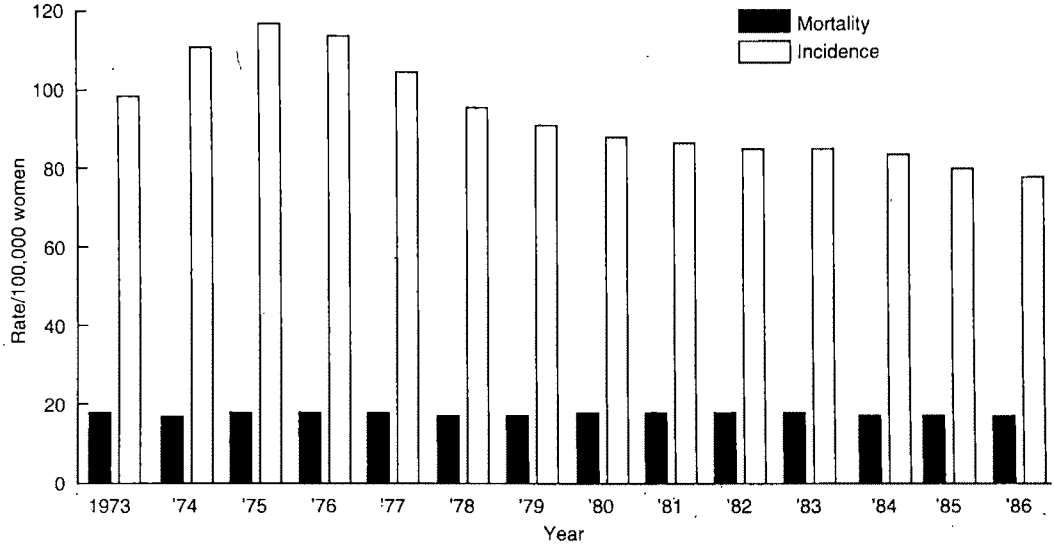


Fig. 3. Mortality caused by and incidence of endometrial cancer in white women in the United States aged more than 50 years, 1973 to 1986. (Source of data: Ries et al.[32])

estrogens have an increased risk of endometrial cancer that depends on the current dose of estrogen (the higher the dose, the higher the risk), the cumulative dose, and the duration of use; the association is consistent whether the study design is prospective or retrospective and is not explained by confounding variables. In women who have used estrogens in the past, the risk of endometrial cancer decreases with increasing time since past use.

Although the relative risk of being diagnosed with endometrial cancer is increased substantially in women who use estrogens (Fig. 2), a part of this increase—perhaps the main part—may be a result of diagnostic bias. Endometrial tumors diagnosed in women who use estrogen are of a lower grade and stage than those in women who do not use estrogen, and the 5-year survival rate (92%) is significantly higher than in women who do not use estrogen (68%).[33, 34] Furthermore, although something of an "epidemic" of endometrial cancer occurred in the late 1960s and early 1970s, and although this declined after 1975 when sales of estrogen were reduced, no changes occurred in the mortality rate caused by the disease over time (Fig. 3). That is, the "epidemic" may have been an artifact, a result of a tendency on the part of pathologists to classify borderline cases of endometrial cancer as malignant if the woman was taking estrogen.

If a causal relationship exists between estrogen use and the excess of endometrial cancer diagnosed in women who use estrogen (and most epidemiologists conclude that such a relationship exists), what public health consequence would result if the mortality rate in each diagnosed woman who uses estrogen was the same as in women who do not use estrogen in whom endometrial cancer is diagnosed? Henderson et al.[29] assumed such a "worst case" approach when they calculated the risk of death that would occur in women who use estrogen replacement therapy. They concluded that among women in the United States aged 65 to 74 years, endometrial cancer would add 26 per 100,000 deaths annually. This number must be balanced against the estimated benefit of 284 per 100,000 deaths from coronary artery disease that are prevented in the same women.

Current knowledge assumes that mortality from endometrial cancer can be redued by an increase in clinical screening and surveillance and that its incidence can be reduced by lowering the rate of obesity. However, compelling evidence shows that the addition of progestins to estrogen replacement therapy is even more useful. Progestin can effectively reduce the occurrence of adenomatous endometrial hyperplasia; at higher doses it can cause regression of preexisting hyperplasia and even of some well-differentiated carcinomas in some patients.[8] Women who take progestins with estrogen are at no increased risk of developing endometrial cancer compared with postmenopausal women who do not take estrogen. Whether the risk is reduced below the risk of an untreated population is unclear. One study[35] suggested this, but more recent work[36] indicates that progestins return the risk of endometrial cancer to that found in women who do not

Table I. Changes in mortality in women aged 65-74 years who receive estrogen or estrogen and progestin

	Change in mortality (per 100,000 women)	
	Estrogen	*Estrogen + progestin*
Hip fractures	−33	−33
Coronary artery disease	−284	−184
Cerebrovascular accident	−49	−32
Endometrial cancer	+26	0
Breast cancer	+38	+38
Total		
With uterus	−302	−211
After hysterectomy	−328	−211

*Modified from Henderson et al. Fertil Steril 1988;49 (suppl):9S-15S. Reproduced with permission of The American Fertility Society.

use progestins. Thus there is a consensus that patients who have an intact uterus should be given progestins in addition to estrogen replacement therapy.[37] The most effective type and dose of progestin and the optimal regimen are still under investigation.[38] No rationale exists for giving progestins to women who have had a hysterectomy.

Breast cancer: Benefit or risk of estrogen?

Approximately 10% of women in the United States will develop breast cancer during their lifetime. The incidence increases with age from 27 per 100,000 at age 30 to 34 years to 422 per 100,000 at age 70 to 74 years. At the time of diagnosis these women experience a 25% reduction in their 5-year survival rate.[32] The National Institutes of Health[32] have estimated that in the United States in 1991 175,000 new cases developed and approximately 44,500 deaths were caused by breast cancer. For women aged 65 to 74 years, the annual mortality caused by breast cancer is 102 per 100,000 women.[29]

Risk factors for postmenopausal breast cancer have been identified as obesity, late age at menopause, late age at first full-term pregnancy, a family history of breast cancer or benign breast disease, and perhaps diet and early age at menarche.[39] A large body of epidemiologic and laboratory evidence links estrogens to breast cancer.[40]

The relationship between breast cancer and estrogen replacement therapy in postmenopausal women is controversial: to date no consensus has been reached, and even the authors of different meta-analyses have reached contradictory conclusions.[41, 42] It is clear, however, that the change in risk caused by estrogen, if any, is quite different from that pertaining to endometrial cancer (Fig. 2). The association between estrogen replacement therapy and endometrial cancer is strong and consistently observed in virtually all studies, but the association between estrogen replacement therapy and breast cancer is weak and inconsistent. Whereas latency and recency of estrogen use affect the risk of developing endometrial cancer, these factors have not been clearly demonstrated in breast cancer. Estrogens have not been associated with any change in the risk of developing breast cancer in women who have taken estrogens for less than 5 years[42, 43] or in women who use conjugated estrogens at a dose of 0.625 or less.[43] Risks seem to be slightly higher in studies conducted in Europe, where synthetic estrogens are used, than in studies conducted in the United States, where most of the estrogen used is conjugated equine estrogen.

On the other hand, the recent meta-analysis by Steinberg et al.[42] has suggested an effect of estrogens in very long-term users; the risk is increased by about 30% after 15 years of use. The same analysis suggested that estrogen replacement therapy might provide a significantly greater relative risk for women who have a family history of breast cancer than for women who do not have such a family history; however, most of the studies on which this conclusion was based used hospital controls, so the finding is difficult to evaluate. The effects of estrogen appeared to be unaltered by parity, benign breast disease, or age at first pregnancy.[42]

Few data on the effects of progestins on breast cancer are available. Studies from Sweden,[44] Denmark,[45] and the United States[46] have shown no evidence for a protective effect of progestins on breast cancer. On the contrary, the relative risk may be slightly higher after several years of combined therapy than after estrogen replacement therapy alone.

One issue that clouds the interpretation of studies of breast cancer and postmenopausal hormone therapy is whether women who use estrogen are screened more frequently and are thus more likely to have their cancer diagnosed early. Evidence for this is controversial. Women who use estrogen are certainly concerned about the risk of breast cancer.[47] Breast tumors diagnosed in women who use estrogen are smaller[48] and carry a better prognosis,[49] which strongly suggests surveillance bias.

Although it is not clear whether the relationship between estrogen and breast cancer is a causal relationship, Henderson et al.[29] conducted a "worst case" analysis to calculate the effect on mortality if such an effect were established. They estimated that estrogen replacement therapy might add about 39 per 100,000 deaths annually in women in the United States aged 65 to 74 years. This must be balanced against the estimated 284 per 100,000 deaths from cardiovascular disease that are prevented in women who receive estrogen replacement therapy.

Balancing the benefits and risks of estrogen therapy

In 1988 Henderson et al.[29] reviewed the pertinent literature on mortality from osteoporotic hip fractures, endometrial cancer, breast cancer, and ischemic heart disease in women aged 65 to 74 years and calculated the hypothetical public health impact of postmenopausal estrogen use. Data on mortality from stroke and revision of the breast cancer data were incorporated into a subsequent analysis (Table I). Annual mortality caused by breast cancer and endometrial cancer would be 38 per 100,000 and 26 per 100,000 higher, respectively, among women who use estrogen versus women who don't use estrogen. Thus total mortality from the two causes that represent the main risks of unopposed estrogen use would be 64 per 10,000 higher in women who use estrogen. On the other hand, mortality caused stroke, osteoporotic hip fractures, and ischemic heart disease would be decreased by 49 per 100,000, 33 per 100,000, and 284 per 100,000, respectively. Thus total mortality from these three major benefits of estrogen would be 366 per 100,000 lower in women who use estrogen. Using this formula, Ross et al.[50] concluded that the predicted annual reduction in mortality among 65- to 74-year-old women who use estrogen was 302 per 100,000 in women who have not had hysterectomies and 328 per 100,000 in women who have had hysterectomies.

A similar formula was used to calculate the difference between mortality in women who use estrogen alone and women who use a combination of estrogen and progestin. This estimate showed that mortality from cardiovascular disease would be lower with estrogen alone than with estrogen and progestin, but that mortality from endometrial cancer would be slightly higher with estrogen alone. Mortality from hip fractures and breast cancer was assumed to be similar with both regimens. These calculations estimated that the addition of progestins to estrogen replacement therapy would reduce the total annual decrease in mortality among women from 302 to 328 per 100,000 to 211 per 100,000 women.

In postmenopausal women mortality from cardiovascular disease is many times higher than mortality from breast cancer,[51] and this difference increases with increasing age. Although replacement therapy may involve an increased risk of breast cancer, this risk is quite small. It is strongly outweighed by the beneficial effects of therapy in preventing cardiovascular disease and osteoporosis.

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The effect of sex steroids on the skeleton in premenopausal women

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Whereas peak bone mass is genetically determined, the skeletal growth, maturation, and maintenance required to reach this peak may be influenced by physical activity, ovarian function, and nutrition. Estrogen deficiency at menopause leads to increased skeletal remodeling and loss of bone mass, which can result in osteoporotic fractures. Entering menopause with low bone mass is a risk factor itself, because bone mass predicts future risk of fracture. The administration of exogenous steroids can reverse the setting of the "mechanostat" to lower levels after ovarian dysfunction, although it is unclear whether oral contraceptives can modify bone mass in the ovulatory premenopausal woman. Our recent data suggest that the use of steroidal hormones to treat menstrual irregularity (presumably relative to ovarian dysfunction), as opposed to fertility control, is more likely to positively affect bone mass. It is even less clear whether hyperestrogenic states (e.g., pregnancy) affect the skeleton. Recent evidence from bone mass changes that occur in women with ovulatory cycles and inadequate luteal phase function suggests that progestins may also influence skeletal metabolism. (Am J Obstet Gynecol 1992;166:1993-6.)

Key words: Peak bone mass, ovarian function, osteoporotic fractures

Bone mass or density in adult life is the algebraic sum of the amount of bone mass accumulated during growth and its subsequent alteration by a variety of factors that change skeletal behavior and modify bone mass. The best known factor is cessation of ovarian function at menopause, which increases skeletal remodeling and accelerates the rate of bone loss.[1-6] The consequence—increased risk of osteoporotic fractures among postmenopausal women—is equally well known. Increasing evidence indicates that entering menopause with low bone mass is itself a risk factor, because bone mass at any time can predict future fracture risk. To date comparatively little attention has been given to those factors that might modify skeletal mass in premenopausal women and alter their risk of osteoporotic fracture before they enter menopause. Onset of menopause would then become a signal for the physician to evaluate the patient's potential risk of fracture. This review evaluates the factors that might change skeletal mass and thereby alter skeletal integrity in women before they reach menopause.

Peak bone mass

Although the amount of bone in the skeleton at the end of linear growth is primarily genetically determined,[6] the potential to reach peak bone mass may be modified by several factors. Many of these factors are similar to those that are known to modify skeletal mass in adult life, including nutrition, physical activity, and intercurrent illness. The importance of these factors has been determined mainly by studying the effects of extremes. For example, paralysis during childhood leads to development of bone mass that, while adequate for the stress placed on the skeleton in a paralyzed person, is clearly inadequate to withstand the stresses that might be placed on it during normal activity.[7] Extremes of nutritional aberration, such as anorexia and bulimia, deprive the skeleton just as they deprive the remainder of the body of essential nutrients.[8, 9] Malabsorption syndromes effect the same process.[10]

In growing children the roles of nutrition and a normal life-style in modifying peak bone mass have been inadequately studied. Preliminary data from one study[11] suggest that calcium intake increases bone mass in teenagers. In this study the effects of calcium supplements that were given to one twin of a series of monovular twin pairs were examined. The findings reveal greater skeletal mass in the twins who received supplementation. However, it is as yet unknown whether the subjects who have faster accretion will subsequently develop maximal (or peak) skeletal mass greater than that developed by the nonsupplemented twin. Thus the study will be incomplete until all twins have reached peak skeletal mass.

The extent to which peak skeletal mass depends on physical activity within the normal ranges for growing children and teenagers is also unclear.[12] Again, this is-

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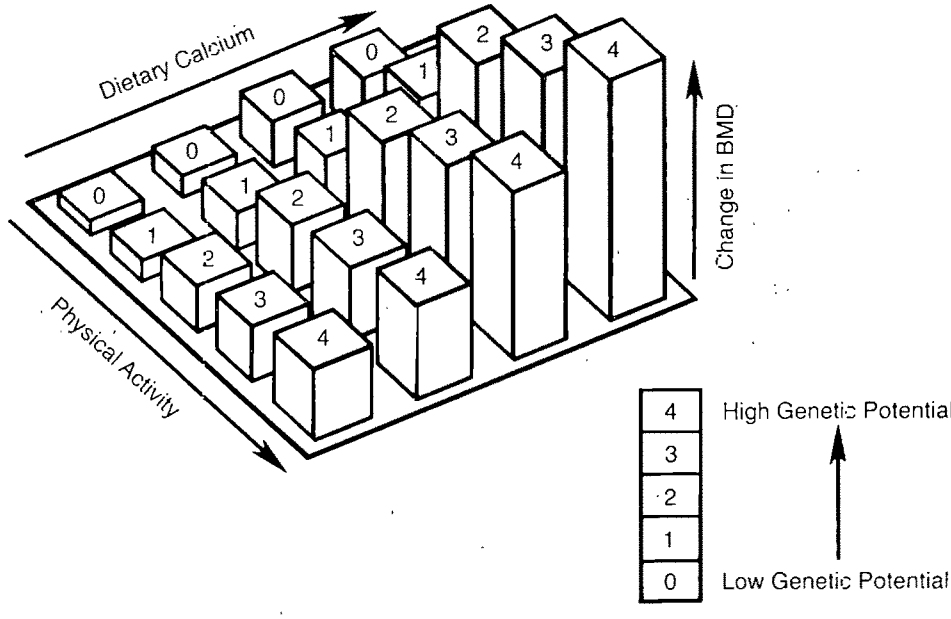


Fig. 1. Premise for interactions between various factors that affect the skeleton. *BMD*, bone mineral density. (From Kelly PJ, et al. Osteoporosis Int 1990;1:56-60. Reprinted with permission.)

sue has been inadequately studied, and it would be a difficult study to design and complete. Experience from adult studies is mixed and depends on the stage at which the patient was examined. Given this, and the fact that the processes of skeletal modeling and linear growth proceed in tandem in the young skeleton, it appears likely that changes in physical activity would cause greater changes in skeletal turnover at this stage of life than at an older age. This hypothesis requires further testing.

The capacity to achieve peak skeletal mass probably requires the development of adequate endocrine function of the ovaries or testes. Evidence again is obtained from the study of extreme situations. Patients who have ovarian agenesis (Turner's syndrome) have low bone mass and high predisposition to osteoporotic fracture.[13, 14] Patients who have Klinefelter's syndrome also have lower than normal bone mass.[15] In addition to the effects of the failure to develop mature ovaries, as seen in ovarian agenesis, there may be additional effects on the skeleton because linear growth is reduced and bone mass may be less than average before the normal age of menarche.[16]

Bone mass reduction

Some data indicate that age at menarche may be a determinant of adult bone mass, but further study is required because all the data do not agree.[17] Ovarian function after menarche may also control skeletal status.[18] Several studies[19, 20] have shown that a reduction in bone mass accompanies loss of ovarian function in premenopausal women. Women with exercise-induced amenorrhea have reduced bone mass compared with those who exercise equally but have intact ovarian function.[19, 21-25] However, most studies also indicate that bone mass is greater in women who exercise than in sedentary women who have normal ovarian function. In this circumstance the degree of reduction in mass is related to the duration and extent of ovarian insufficiency.[12] Thus moderate oligomenorrhea is associated with a more modest reduction in mass than complete loss of ovarian activity for a similar duration. It is unclear to what extent these relatively small reductions in bone mass increase the risk of osteoporotic fractures among these women after menopause. When the criterion of bone mass is strictly used—because bone mass is still above the average for healthy, premenopausal women—it does not appear to be a problem. However, the risk of injury and peripheral fractures (so-called stress fractures) appears to be increased among amenorrheic athletes,[20, 26] and therefore further study of this issue is warranted.

Other causes of reduced ovarian function also result in low bone mass. Hyperprolactinemia, for example, causes low bone mass only when it is sufficiently severe to reduce the function of the ovaries.[27] The effects of anorexia and bulimia are compounded by the accompanying loss of ovarian function. Recent evidence suggests that even modest alterations in ovarian function can produce significant changes in the skeleton.[28] The increasing frequency of impaired progesterone production in the second half of the normal ovarian cycle (often called inadequate luteal phase) apparently results in bone mass reduction. This could account for

the variable results seen in cross-sectional studies of bone mass in premenopausal women, in which some studies found evidence of premenopausal bone loss in the spine or femoral neck.[29-31] Again, further evaluation of this issue is necessary. Although nulliparity is often considered a risk factor for postmenopausal osteoporosis, study results are not conclusive.[32] A high frequency of impaired ovarian function that results in infertility might account for the relationship. If nulliparity occurred because of impaired fertility of ovarian origin in the population examined, then that population might be expected to show a higher risk for osteoporotic fracture. This hypothesis remains untested. As ovarian function begins to decline with age, bone loss ensues and the menopausal inflexion in bone mass begins.

The various factors that affect the skeleton are likely to interact. For example, loss of ovarian function complicates the basic dietary problems found in anorectic patients. The theoretic premise for these interactions has been described[33] (Fig. 1). As much as 40% of the bone density variance found in premenopausal women may be explained by variations in physical stress.[34] Differences in calcium nutrition contribute 40% of the variance in bone density of the femoral neck, at least in men.[35] It seems likely that these effects are independent. In our study of women aged 25 to 35 years, all of whom had normal ovarian function, we found that vertebral bone density was higher in situations in which high calcium intake was combined with high levels of physical activity.[36] Again, interactions of life-style, nutrition, and ovarian function have been inadequately studied.

Conclusions

Although the amount of bone in the adult skeleton, often called peak bone mass, is primarily genetically controlled, the potential to acquire the genetically programmed amount can be modified by various factors. These factors are similar to the extrinsic factors that are known to modify skeletal mass in mature persons. However, the magnitude of the effects have been inadequately studied. This may be important because the effects produced, for example, by altering diet, life-style, or endocrine function, may be greater or more important in the younger skeleton that in the older skeleton.

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The role of progestins in hormone replacement therapy

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Long-term estrogen replacement therapy prevents osteoporosis and reduces the risk of cardiovascular disease in postmenopausal women. Progestins are used to prevent endometrial hyperplasia and carcinoma but should not be prescribed for women who have had hysterectomies. Doses that decrease mitotic activity are sufficient. Recent data suggest that a continuous combined regimen of estrogen and progestin may increase bone mass in women who have established osteoporosis. Some progestins may oppose the beneficial effects of estrogen on the cardiovascular system in some women. Critical cardiovascular effects of progestins include a reduction in the serum level of high-density lipoprotein cholesterol and a direct effect on arterial tone, which may be mediated by an attenuation of prostacyclin production and other factors. Therefore it is prudent to prescribe the lowest effective dose of a progestin that demonstrates the least metabolic impact. The dose and type of progestin should be balanced with the estrogen component so that the estrogen-dominant metabolic effects prevail. With this approach the difference in mortality rates between women who use unopposed estrogen and women who use estrogen-progestin therapy will be minimized and will make the appropriate sequential addition of progestin an option in postmenopausal hormone replacement therapy. (Am J Obstet Gynecol 1992;166:1997-2004.)

Key words: Progestins, hormone replacement therapy, menopause, estrogen

It is well established that the adverse effects of the cessation of ovarian estrogen secretion in postmenopausal women can be ameliorated or reversed by estrogen replacement therapy. The effects of estrogen on osteoporotic bone fracture and cardiovascular disease are of major clinical importance. Estrogen prevents the accelerated loss of bone mass[1] and thereby reduces the risk of osteoporotic fracture by 50% to 60%.[2, 3] Estrogen also protects against cardiovascular disease and coronary heart disease, in part because of its beneficial effect on lipoprotein metabolism.[4] Atherogenic changes in the lipid profile occur as estrogen levels are reduced in early menopause; serum concentrations of total cholesterol and low-density lipoprotein cholesterol (LDL-C) increase, and these are the principal findings. However, serum triglyceride levels have also been found to increase, and high-density lipoprotein cholesterol (HDL-C) levels may also decrease.[5] Exogenous estrogen reverses these changes[6] and decreases the relative risk of coronary heart disease in postmenopausal women to 0.5 compared with women who do not use estrogen.[7, 8]

On the other hand, postmenopausal women who receive unopposed estrogen therapy have an increased incidence of hyperplasia, which may progress to endometrial adenocarcinoma under the continued influence of estrogen.[9] This association of unopposed estrogen and endometrial cancer has been demonstrated in 17 of 19 case-control studies.[4] Compared with the risk in women who do not use estrogen, the relative risk of endometrial cancer is 2.1 after 2 to 5 years of estrogen use and increases to 3.5 after 6 years of use.[10] However, because vaginal bleeding is an early symptom of this disease, there is usually an early diagnosis and a favorable prognosis.

Epidemiologic and histologic studies have clearly demonstrated that the addition of progestins to estrogen replacement therapy not only decreases the risk of endometrial cancer[11, 12, 13] but can also reverse established endometrial hyperplasia to normal endometrium.[14] However, progestins are not without their own untoward side effects. Although it is known that progestins attenuate the estrogen-induced elevation in HDL-C levels that are associated with decreased risk of cardiovascular disease,[15-17] it is unlikely that changes in the lipoprotein profile are solely responsible for the cardioprotective effects of estrogen. Estrogens may have other direct or indirect effects on the cardiovascular system, and some of these effects may be inhibited by progestins as well.[18, 19] Because the option of adding progestins to a postmenopausal replacement hormone regimen has significant public health consequences, data that bears on the effect of progestins must be reviewed from the perspective of the pharmacokinetic,

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pharmacodynamic, biochemical, and histologic action of various progestins with regard to their clinical impact. Differences in the activities of specific progestins, minimal effective doses, and optimal duration of therapy will be discussed so their role in postmenopausal hormone replacement therapy can be reevaluated under more appropriate conditions of use.

Role of progestins in endometrial cancer

The biochemical effects of estrogen that are associated with histologically confirmed endometrial hyperplasia and the attenuating effects of progestins that result in a secretory-phase endometrium in postmenopausal women were elucidated by Whitehead et al.[20] These investigators demonstrated that the potent stimulation of conjugated equine estrogens (Cee) (either 0.625 or 1.25 mg/day) that resulted in a proliferative-phase endometrium were associated with increases in (1) deoxyribonucleic acid synthesis and mitotic activity (as reflected in the epithelial glandular labeling index), (2) levels of nuclear estradiol receptors, and (3) levels of soluble progesterone receptors and decreases in estradiol and isocitric dehydrogenase activities. Progestational stimulation that resulted in a secretory-phase endometrium occurred after the addition of norethindrone (NET) (1.0, 2.5, or 5.0 mg daily) or norgestrel (150 or 500 μg daily) for the last 10 days of each month. This was associated with significant reduction of the labeling index and nuclear estradiol receptors to within or below the secretory-phase range and significantly increased activity of estradiol dehydrogenase and isocitric dehydrogenase. This showed that these progestins were capable of biochemically protecting the endometrium against the proliferative effects of estrogen.

This study was continued and expanded by King and Whitehead,[21] who analyzed the biochemical and morphologic effects of 6 to 12 days of treatment with three dose levels of each of five orally administered progestins on the endometrium of estrogen-treated postmenopausal women. After 6 days of progestin administration, the biochemical effects were assessed by means of the above analytes, and the morphology was evaluated by means of the secretory histologic features, subnuclear gylcogen content, presence of giant mitochondria, and appearance of the nucleolar channel system. From these data the oral dose of each progestin required to elicit morphology and biochemistry comparable with a secretory-phase endometrium was determined (Table I). After relating the effects of the progestins to a standardized value for NET and converting the potency of dl-norgestrel to that of levonorgestrel for comparative purposes, it was concluded that levonorgestrel is approximately eight times as potent as NET, with medroxyprogesterone acetate (MPA) and progesterone demonstrating 9% and 0.2% the activity of NET, respectively. Estimations of the oral potencies of these progestins were in general agreement with those calculated by means of different methods. However, although these data are a valuable contribution to our understanding of relative potencies, the potency of MPA may have been underestimated because of pharmacodynamic and bioavailability problems with the MPA preparation that was studied.

The dose and duration of progestin treatment are critical to its effect in ameliorating estrogen-induced hyperplasia. These data were obtained on the basis of biochemical and morphologic analyses after only 6 days of progestin administration, although Paterson et al.[18] had previously shown that the effect of progestins in attenuating the effects of estrogen on the endometrium was optimal if the progestin was administered for more than 10 days per month. On the other hand, Gibbons et al.[22] reported that the relationship between doses of 0.3, 0.625, and 1.25 mg of CEE and the increase in cytosolic estrogen receptor concentration from baseline levels was linear. However, in women who use daily doses of either 0.3 or 0.625 mg of CEE, doses of 2.5, 5, and 10 mg of MPA were all equally successful in reducing estradiol receptor concentrations to pretreatment levels, whereas if the CEE dose were raised to 1.25 mg/day, only 5 or 10 mg of MPA was effective in reducing estradiol receptor concentrations (Fig. 1). These investigators also demonstrated a nonparallelism between the biochemical and the morphologic effects of progestins on the endometrium: although 5 and 10 mg of MPA had identical biochemical effects, only 10 mg of MPA produced a morphologically secretory endometrium. They suggested that eliciting secretory changes in the endometrium is probably unnecessary for the inhibition of hyperplasia and considered it more appropriate to strive for a balance of estrogen and progesterone doses that decrease mitotic activity without inducing full secretory changes. This balance has the further advantage of reducing bleeding.

Effect of progestins on lipid metabolism

As a consequence of menopause, serum levels of total cholesterol, LDL-C, and triglycerides increase significantly and HDL-C decreases significantly, which contributes to a more atherogenic lipid profile.[5,23] The cardiovascular benefits of estrogens have been demonstrated in epidemiologic studies, which show that estrogen replacement therapy decreases the relative risk of death from myocardial infarction to 0.59 and the relative risk of coronary heart disease to 0.5 compared with the relative risk of 1.0 for nonusers.[7,24] The

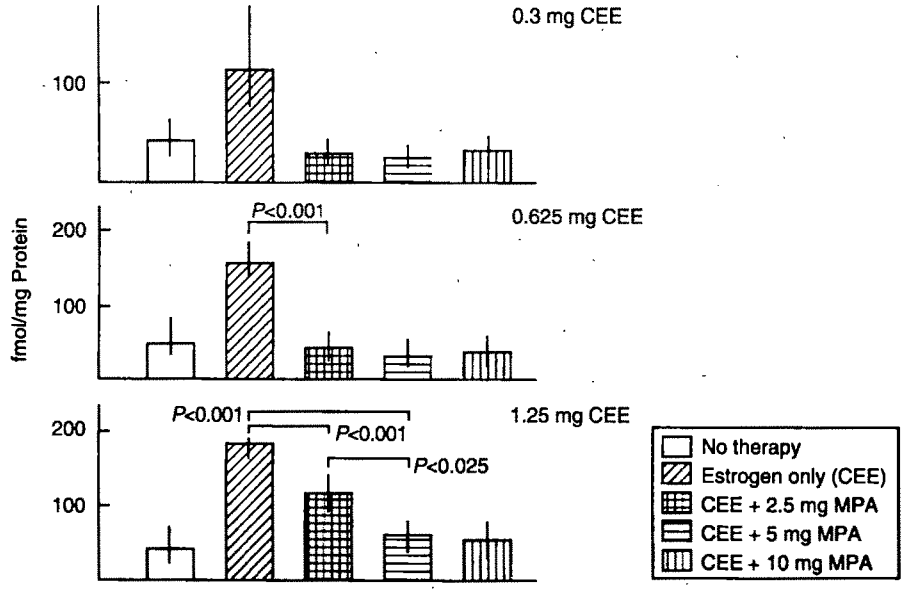


Fig. 1. Concentrations of cytosolic estrogen receptor in three groups of postmenopausal women receiving various therapies. (From Gibbons WE, et al., AM J OBSTET GYNECOL 1986;154:456-61.)

Table I. Oral doses of progestins (mg) required to elicit premenopausal secretory changes

Progestin	*Nuclear estradiol receptor*	*Estradiol dehydrogenase*	*Morphologic value*	*Consensus**
ET	<.35	<.35	.35	.35
dl-Norgestrel	.15	<.075	.075	.075
MPA	<2.5	<2.5	>10	5
Progesterone	200	250	>300	200

From King RJB, Whitehead MI. Fertil Steril 1986;46:1062-6. Reproduced with permission of The American Fertility Society.

*Dose tested (mg) that best fits all the parameters analyzed.

presumption has been that alterations in lipid metabolism alone are responsible for the observed decrease in cardiovascular risk that is associated with estrogen replacement therapy in postmenopausal women. However, a residual 30% reduction in the mortality rate for cardiovascular disease occurred after HDL-C was factored into the original multivariate analysis of the Lipid Research Clinics study data. These earlier data that suggest that other factors are involved in the cause of cardiovascular disease have been further refined by newer data from Bush et al.,[25] which now suggest that the HDL-C effect may only explain 30% to 40% of the benefit.[25, 26]

Extensive studies of progestins as components of oral contraceptives have clearly demonstrated that progestins attenuate the estrogen-induced increases of HDL-C and HDL_2-C in women of reproductive age and that their activity is related to the dose and androgenicity of the progestin.[27-30] Therefore when progestins were added to the estrogen replacement therapy to decrease the risk of endometrial hyperplasia and cancer, the effect of the added progestin on the lipid profile was investigated. One study revealed that sequential doses of norgestrel (250 μg) and to a lesser extent MPA (10 mg) reversed the elevated levels of HDL-C and HDL_2-C in postmenopausal women who were treated with 2 mg of estradiol valerate per day.[15] Sherwin and Gelfand[17] determined that the addition of progestin results in a dose-related reduction in the beneficial effect of estrogen on plasma lipids. HDL-C levels were significantly above pretreatment values in postmenopausal subjects who received 1.25 mg of CEE with or without the addition of MPA (5 mg) sequentially for 1 year (Table II). The HDL-C/LDL-C ratio, which is considered a reliable antiatherogenic index, also increased above pretherapy levels in the high-dose CEE group, irrespective of the addition of MPA. A recent review of the effects of postmenopausal estrogen with and without the addition of progestin on serum lipids shows that estrogen-induced HDL-C increases are attenuated

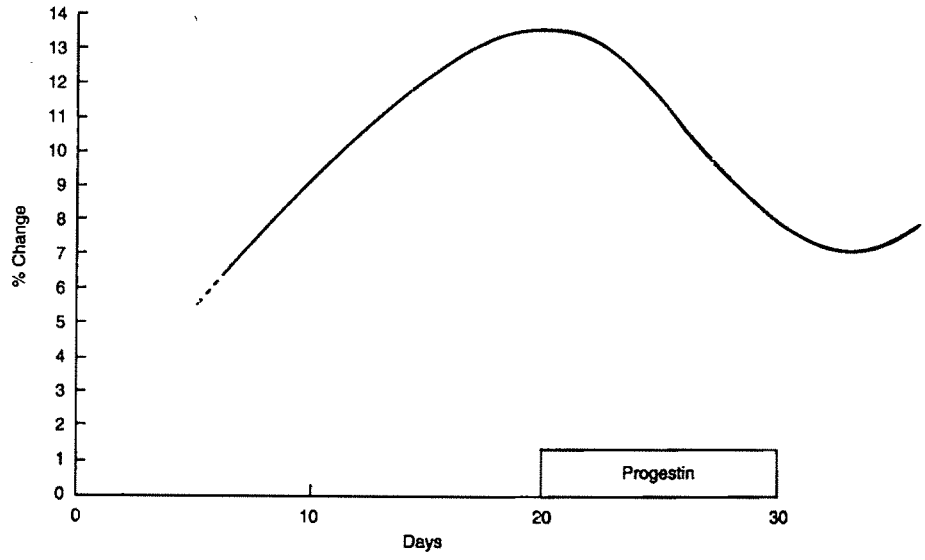


Fig. 2. Attenuation of HDL-C by progestin. Adapted from responses of 0.625 mg CEE, 5 mg MPA, and 1 mg NET. (Modified from Jensen J, et al. Br J Obstet Gynaecol 1986;93:613-8, and Lobo RA. Effects of hormonal replacement on lipids and lipoproteins in postmenopausal women. J Clin Endocrinol Metab 1991;73:925-30. Reprinted with permission of The Endocrine Society.)

Table II. Effect of CEE* and MPA on HDL-C and LDL-C (prospective 1-year study)

	% change (mo 12)		
	HDL-C	*LDL-C*	*HDL-C/LDL-C*
CEE 0.625 mg	↑ 13.7	↓ 0.3	↑ 13.6
CEE 0.625 mg + 5 mg MPA	↑ 4.3	↓ 3.4	↑ 7.3
CEE 1.25 mg	↑ 19.0	↓ 17.6	↑ 43.0
CEE 1.25 mg + 5 mg MPA	↑ 13.4	↓ 8.3	↑ 24.0

Modified from Sherwin BB, Gelfand MM. Obstet Gynecol 1989;73:759-66. Reprinted with permission from the American College of Obstetricians and Gynecologists.

*CEE days 1 to 25; MPA days 15 to 25 (sampling days 18 to 20).

more by levonorgestrel (0.25 mg) than by MPA (10 mg).[31] The data of Jensen et al.[32] and Lobo,[33] however, showed that HDL-C levels increase only 13% to 14% in response to 0.625 mg of CEE and attenuation by either 5 mg of MPA or 1 mg of NET is clinically evident only during the 10 to 12 days of progestin administration. The HDL-C levels were not reduced to baseline levels at any time during the treatment cycle and were increased when progestin administration was discontinued (Fig. 2). Finally, the route of administration plays a role in the effect of the progestin. Estradiol with or without NET acetate was administered to postmenopausal women by the transdermal route, thus eliminating the first-pass effect on the liver. Preliminary results indicated that the transdermal patch, which delivered 0.2 to 0.3 mg of NET acetate per day, had no significant adverse effect on serum lipid levels.[34]

Thus evidence shows that the degree of attenuation of lipid effects by the addition of progestins to estrogen replacement therapy depends on the type or androgenicity of the progestin, the doses of both the estrogen and the progestin, the regimen, and the route of administration.

Other biochemical factors contributing to cardiovascular disease

The effect of progestins on the risk of cardiovascular disease, however, is clearly not restricted to their impact on lipid metabolism. Although the relative importance of other biochemical factors is unclear, these factors undoubtedly have a role in progestin's attenuation of the cardioprotective effect of estrogen and therefore require further elucidation.

It is known that estrogen increases uterine blood flow in the animal model[35] and that progesterone (administered either intramuscularly or by intraarterial infusion) inhibits this response.[18, 36] It has been speculated that the partial depletion of cytoplasmic estrogen receptors, a known effect of progestins that reduces the sensitivity of the uterus to subsequent estrogen action,[37] may contribute to the mechanism of this inhibition.[18] This suggests that the depletion of estrogen receptors by progestins plays a role in cardiovascular disease by regulating blood flow.

Progestins may also contribute to thrombosis by shifting the balance in prostaglandin synthesis. Prostacyclin

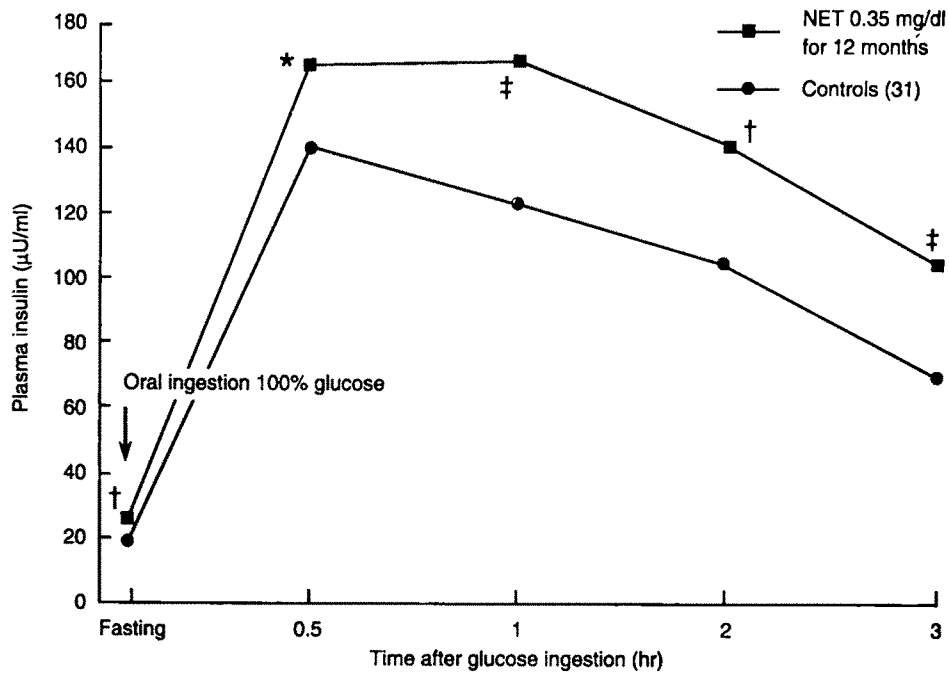


Fig. 3. Effect of NET on carbohydrate metabolism. $*p < 0.05$; $\dagger p < 0.01$; $\ddagger p < 0.001$. Conversion factor for insulin from conventional (μU/ml) to Système International units (pmol/L) is 7.175. (From Spellacy WN, et al. Obstet Gynecol 1975;46:560-3. Reprinted with permission from The American College of Obstetricians and Gynecologists.)

is a potent inhibitor of platelet aggregation and causes vasodilation, and thromboxane is its endogenous antagonist. Therefore a balance between the two may regulate vascular tone and platelet vascular interaction.[38, 39] When progestin is added to estrogen replacement therapy this balance shifts slightly toward thromboxane dominance, which theoretically increases the risk of thrombosis.[19, 40]

Whereas it is clear that both estrogens and progestins influence vasomotor tone, blood coagulation, and blood flow, the precise mechanisms involved require further investigation. Sarrel[41] has recently reviewed the current status of knowledge in this field.

Progestins and carbohydrate metabolism

Studies of the effects of oral contraceptives have demonstrated that some combinations of estrogens and progestins affect carbohydrate metabolism. Therefore it is important to recognize not only that the potential risk of diabetes is increased but that hyperinsulinemia is an independent risk factor for cardiovascular disease,[42] with the risk of myocardial infarction in diabetic women twice that of nondiabetic women.[43] In one study combination oral contraceptives increased glucose levels 43% to 61% and insulin levels 12% to 40% above baseline levels in an oral glucose tolerance test, although progestin-only formulations or combined oral contraceptives with desogestrel or low-dose NET as their progestin component had only minor effects on these parameters.[44] In another study, however, NET administered for 12 months at the same dose (0.35 mg) increased plasma insulin levels significantly at each oral glucose tolerance test sampling, whereas blood glucose values remained unchanged, which suggests insulin resistance (Fig. 3).[45] Although the types and doses of estrogen and progestin may differ in hormone replacement therapy, it is probable that the mechanisms by

Table III. Theoretic change in mortality rate with progestins (per 100,000 women aged 65 to 74 years)

	Estrogen alone		*Estrogen + progestin*	
	RR	*No. of women*	*RR*	*No. of women*
Ischemic heart disease	0.50	296	0.57	255Δ+41
Osteoporosis	0.40	33	0.40	33Δ 0
Breast cancer	1.1	10	1.1*	10Δ 0
Endometrial cancer	6.0	26*	1.0*	0Δ−26

Unpublished data, 1990.
RR, Relative risk.
*Data unclear.

which they exercise their effects on carbohydrate metabolism are similar.

In summary the addition of progestins to estrogen replacement therapy can have adverse cardiovascular effects by means of the impact of progestins on lipid metabolism, blood flow, and carbohydrate intolerance. To overcome these effects and maintain an overall favorable cardiovascular profile, it is advisable to use sufficient estrogen and minimal progestin. This can best be accomplished by individualizing dose levels according to each patient's biochemical profile.[46]

Effects of progestins on osteoporosis and breast cancer

Few studies have been performed on the long-term effects of hormone replacement therapy on postmenopausal osteoporosis and risk of bone fracture. However, it has been established that postmenopausal bone loss and the risk of osteoporotic fractures can be prevented or greatly reduced by replacement of estrogen alone.[1-3] Some investigators have suggested that progestins alone also protect against osteoporosis.[47, 48] Other studies have shown that estrogens in combination with progestins have an effect on bone similar to that of estrogen alone.[49, 50] A recent study has demonstrated that the daily administration of estradiol (2 mg), NET acetate (1 mg), and calcium (500 mg) significantly increased bone mass in women who had established osteoporosis.[51] Although additional data are needed about the effects of different doses, type of estrogen and progestin, and duration of treatment, this finding holds promise for women who already suffer from postmenopausal osteoporosis.

Ernster et al.[4] reviewed 10 case-control studies of the effect of estrogen on breast cancer and found that the data were inconsistent and controversial. Studies on the effect of estrogens and progestins on breast cancer risk have been conducted in both younger women who use oral contraceptives and in postmenopausal women after combined hormone replacement therapy. Various studies have shown both a protective effect and a detrimental effect of the addition of progestin to estrogen relacement therapy.[52, 53, 54]

The relationship between hormone replacement therapy and breast cancer requires further study before any convincing argument can be made for its benefits and risks.

Psychological effects of mood disturbances

Whereas estrogen has been shown to improve mood and psychological well-being in postmenopausal women, progestins have been shown to have an adverse effect, at least in some women.[17] Again, both the type of dose and duration of progestin use are important variables that contribute to these effects. Therefore reductions in dose and duration of use may be important factors in helping provide an excellent quality of life for postmenopausal women.

Public health impact of adding progestins

Theoretically the appropriate use of progestins in hormone replacement therapy would substantially change the mortality rates in women 65 to 74 years of age.[33] Cumulative mortality data from the Leisure World (Laguna Hills, Calif.) cohort[55] were used to recalculate annualized data to reflect the impact of administering progestins at a lower dose for only 10 days in a 30-day cycle. This recalculation is based on the assumption that the HDL-C effect (which is reduced 50% by progestin) and the changes in blood flow (which serve as a surrogate for the arterial effects of estrogen) only occur for 10 of 30 days during progestin administration. Taken together this accounts for a downward change in the relative risk by 15%. This model suggests that if progestins were appropriately used, the relative risk of death caused by ischemic heart disease would change from 0.50 to 0.57, resulting in an additional 41 deaths per 100,000 women compared with those who use unopposed estrogen (Table III). On the other hand, the relative risk of death caused by endometrial cancer would be reduced from 6.0 to 1.0, which would save 26 lives per 100,000 women. Thus although the use of unopposed estrogen results in a lower overall mortality rate, appropriate sequential addition of low-dose progestins is an option worth considering. Because no firm data are available to date, and on weighing together all the issues, it is my view that progestins should be administered sequentially with use of a lower dose (2.5 to 5 mg of MPA, 1 mg of NET) for only 10 days. Secretory changes of the endometrium should not be a requirement and reductions in endometrial mitotic activity and elimination of hyperplasia should be the focus of treatment. Clearly, as with all hormonal therapy, appropriate monitoring should be carried out, particularly with the use of lower doses of progestins.

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Use of two types of estradiol-releasing skin patches for menopausal patients in a tropical climate

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As part of a program to evaluate nonoral routes for delivery of estrogen replacement therapy at the National University Hospital, Singapore, we assessed two different 50 μg estradiol patches. The first study (patch A) was terminated because of a high rate of skin reactions. Tests of the components of this patch identified the alcohol carrier as the cause of these reactions when combined with an occlusive membrane in the tropical Singapore climate. A subsequent clinical trial with 21 female subjects was undertaken with a different 50 μg estradiol patch (patch B). Twenty patients completed the 6-month study. Patch B was as effective as patch A, caused fewer skin reactions, and adhered better. (Am J Obstet Gynecol 1992;166:2005-10.)

Key words: Transdermal estrogen patch, estradiol, skin reactions

Transdermal patches provide an accepted route of delivery for hormone replacement in Europe and the United States.[1, 2] The advantages of transdermal patches include a controlled, sustained release of estrogen,[3] predictable absorption,[4] lack of gastrointestinal effects,[5] circumvention of first-pass circulation through the liver,[6] lower biliary cholesterol saturation index,[7] and slower metabolism to less-active estrogens,[8] which enables maintenance of therapeutic blood levels with a lower total dose of estrogen than in standard oral regimens (e.g., estradiol valerate, 2 mg/day, or conjugated estrogens [Premarin], 0.625 mg/day). At the same time the ability to relieve menopausal symptoms[9] and to protect against osteoporosis[10] is preserved, with significant beneficial effects on high-density lipoprotein/low-density lipoprotein ratios.[11]

The Menopause Clinic of the National University Hospital, Singapore, has a continuing program to evaluate nonoral routes for estrogen administration, which include subdermal implants, patches, and percutaneous creams. In 1986 the Clinic began an informal trial of 50 μg estradiol patches (Estraderm TTS) from Ciba-Geigy AG (Basel, Switzerland). It quickly became apparent that the rate and severity of skin reactions were much higher than had been reported in other series,[12, 13] and the trial was terminated. Four of the women who had experienced skin reactions underwent skin tests in an effort to ascertain which components caused sensitivity. Finally, a formal clinical trial was undertaken to evaluate a different 50 μg estradiol patch from Ortho-Cilag (Schasshausen, Switzerland). These studies are briefly described.

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Experience with the Ciba-Geigy 50 μg estradiol patch (patch A)

Sixteen women had been recruited for an evaluation of the Ciba-Geigy 50 μg estradiol patch before the trial was discontinued. Eleven of the women were menopausal (amenorrhea >6 months) and five were perimenopausal, with a mean age of 48.7 years. None of the women had had a hysterectomy. All had been receiving another form of estrogen replacement, and 14 were also taking cyclical progestins (Table I). The patients were instructed to apply the transdermal patch anywhere on their bodies except in the breast area. The patches were changed twice weekly and were used continuously during a 2-month period. In addition to the transdermal estrogen, the women were given progestin on days 1 through 12 of each month (14 women received norethindrone, 1.25 mg, 1 woman received norethindrone, 2.5 mg, and 1 woman received medroxyprogesterone acetate, 5 mg).

Serum estradiol levels were measured during the time of patch use in six women. Five had levels within the normal range for the follicular phase (328.6 ± 120.8 pmol/L); the remaining woman had a level of 99.1 pmol/L, which is a suboptimal therapeutic level.[14]

Because all the women had received oral estrogens before entry into the study, they were able to compare the symptomatic relief obtained with the two regimens. Seven women stopped use of the patch within the first 2 months because they believed it provided less symptomatic relief than did the oral preparation, because of skin reactions.

Adverse skin reactions included itching, discomfort, vesicular rash, erythema, edema, induration, and re-



Fig. 1. Severe skin reaction after removal of 50 μg Estraderm patch (patch A).

Table I. Summary of the Ciba-Geigy 50 μg estradiol patch study (patch A)

No. of participants	16
Age range (yr)	44-54
No. who received pretrial HRT	16
No. who received pretrial HRT and cyclical progestins	14
No. who continued at 2/12	4
No. who experienced adverse side effects	
Moderate or severe itching	14
Erythema	9
Pigmentation >4 mo	2
Soggy with perspiration, or after showering or swimming	5
Pain	2
Vesicular eruption	1
No. who experienced patch displacement during 2 mo use	9

HRT, Hormone replacement therapy.

sidual pigmentation. Four of 16 patients experienced severe skin reactions,[15] and all reported some itching and redness that increased with duration of use. Skin marks at the site of severe reactions (Fig. 1) often persisted for several weeks (Fig. 2). Two women had residual pigmentation more than 4 months after removal of the patch.

Some women reported that moisture developed under the patch, which resulted in soggy skin and loss of adhesion. Although the patients were not specifically asked about adhesiveness, case records show that at least nine women mentioned that the patches fell off before the expected 3 to 4 days. Only four women chose to continue use of the patches at the end of the trial, although they would have been provided without charge.

Skin tests of components of the Ciba-Geigy patch

Four of the patients who had experienced cutaneous reactions were recalled for skin tests of components of the patch (Table II). The patches were applied in the outpatient clinic and were left in place for 72 hours. Only patches 1 and 6 provided a combination of ethanol and an occlusive membrane; patch 6 also contained estradiol.

Similar reactions were observed in all four patients but were more severe in two.[16] Erythema and itching were most pronounced with patch 1 but also appeared at the site of patch 6 (Fig. 3). Edema was also seen at the site of patch 1 in three patients. In three of the four women one or more patches became dislodged and had to be reapplied. We concluded that the occlusive element combined with ethanol is associated with adverse skin reactions in the hot, humid Singapore climate. The addition of estradiol appeared to alleviate the reactions.

Clinical trial of the Ortho-Cilag 50 μg estradiol patch (patch B)

This study was part of an international trial of the tolerability, adhesiveness, acceptance, and efficacy of transdermal estrogen patches. The patches were designed to release of 50 μg of estradiol every 24 hours and were changed twice weekly (on Mondays and Thursdays). Patients were recruited according to a strict admission protocol. They were to be 50 years of age or older, to have been amenorrheic for at least 12 months, and to have symptoms that were attributable to estrogen deficiency (not necessarily flushes). In addition, they could not have received any hormone re-

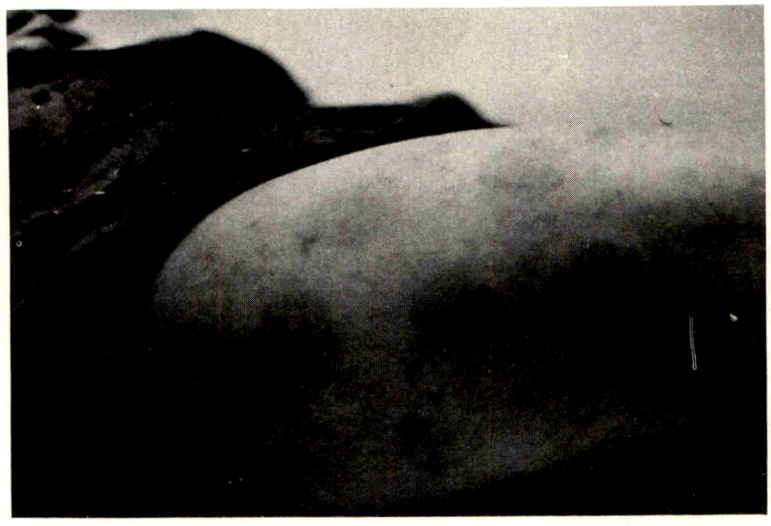


Fig. 2. Persistent skin reactions after removal of 50 μg Estraderm patches (patch A). Because a new patch was applied twice weekly, reactions shown are result of application of patches over 2½ weeks.

placement therapy for at least 2 months before entry to the study, and their uterus had to be in situ. Patients who were allergic to adhesive bandages and those in whom estrogen replacement therapy was contraindicated were excluded.

Each woman was examined in the Menopause Clinic before entering the trial and returned to the clinic once a month for 6 months to complete a detailed questionnaire. At the final visit, 2 weeks after removal of the last patch, each woman completed a questionnaire about acceptability of the patch, and the physician in charge made a global assessment of symptom relief.

Twenty-one women were treated with the Ortho-Cilag 50 μg estradiol patch (patch B). The patches were 16 cm^2 with rounded edges. A monolayered adhesive film that contained the estradiol was located under a transparent occlusive foil backing in each patch. The patches were used for either 3 or 4 days (changed on Mondays and Thursdays) during a 3-week period, followed by a patch-free interval of 1 week in each 28-day cycle. Initially patients were prescribed 0.35 mg of norethindrone on days 10 through 21. Because withdrawal bleeding was minimal or early in most of the cases, this dose was increased to 1.25 mg for 12 days in nine women, with a further increase to 2.5 mg in six women. By the end of the trial 18 of 20 women had withdrawal bleeding, with a mean duration of 5.9 days (range, 2 to 10 days).

This study provided an introduction to estrogen replacement therapy for 14 women, whereas the others had not received treatment for at least 2 months. One patient was erroneously enrolled at the age of 49 years but follow-up was continued according to the protocol. A woman who enrolled as a replacement withdrew in the first week because of a viral illness; at that time she reported that her symptoms of "hotness" were not improved. Thus 20 patients completed the 6-month trial. Their mean age was 53.6 years, with a range of 49 to 59 years (Table III). The mean duration of amenorrhea was 35.8 months (±27.3), and the mean duration of postmenopausal symptoms was 27.6 months (±20.5).

Table II. Summary of components in Estraderm TTS test patch kit

System No.	*Components*
1	TTS placebo (without estradiol) Reservoir with ethanol
2	TTS placebo (without estradiol) Reservoir with methylparaben in water
3	TTS 3M (without estradiol) Nonocclusive Without reservoir
4	TTS 3M (without estradiol) Occlusive Without reservoir
5	TTS PIB (without estradiol) Nonocclusive
6	TTS estradiol, 25 mg Reservoir with ethanol

Eight of 12 patients who had had hot flushes attained complete relief of this symptom by the fourth cycle, and the remaining four women experienced a reduction in intensity of the flushes. The maximum number of flushes per day declined from 25 to 3. The percentage of women who had nocturnal sweating declined from 45% to 30%, and by the sixth cycle only one patient continued to experience this symptom frequently. Similar reductions in depression (45% to 20%), insomnia (65% to 25%), and vaginal burning or itching

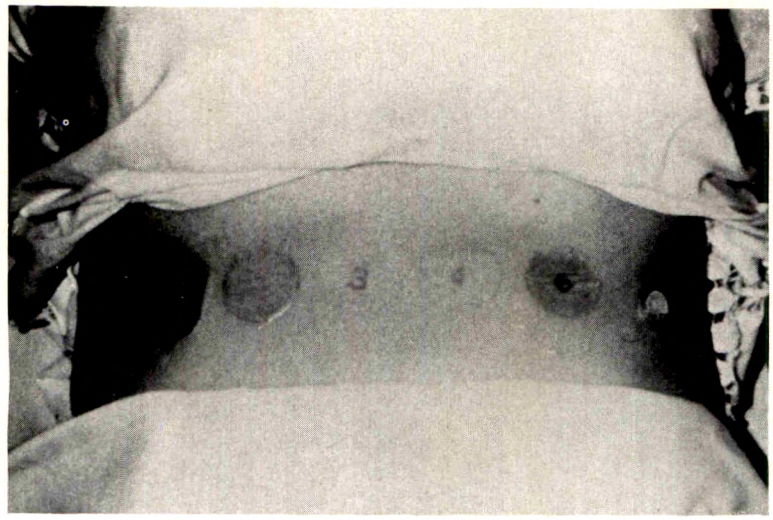


Fig. 3. Sites of application of six individual component patch tests in a patient who had previously experienced adverse reactions with 50 μg Estraderm patches (patch A).

Table III. Summary of the Ortho-Cilag 50 μg estradiol patch study (patch B)

No. of participants	21*
Age range (yr)	49-59
No. with no previous HRT exposure	14
No. with previous HRT exposure (but free of all therapy for at least 2 mo)	7
No. who wished to continue at 6/12	17
No. who experienced adverse side effects†	
Slight itching	10
Erythema	9
No. who experienced patch displacement during 6 mo use	1

HRT, Hormone replacement therapy.

*One patient withdrew in the first week of use because of viral illness.

†Side effects were not consistently recorded and were limited to one cycle or in eight women.

(30% to 10%) were obtained. According to the physician's global evaluation, 16 (80%) of the patients had very good or good overall improvement of menopausal symptoms (Fig. 4). The lack of response in three patients was attributed to minimal improvements in the other symptoms, including fatigue, nervous irritability, headache, dizziness, and palpitations. A slight but significant ($p < 0.001$) fall in both systolic and diastolic blood pressure also occurred (pretrial: 129.5 [±11.4]/81.6 [±7.9] mm Hg; cycle 6: 116.6 [±11.7]/74.0 [±6.7] mm Hg).

The frequency of erythema at the patch site declined from 11 women in the first cycle to 5 women by the sixth cycle; the mean (±standard deviation) duration of erythema was 1.2 (±0.4) days. Edema ($n = 3$) and eczema ($n = 2$) were reported in the first cycle only. Three of the six women who recorded itching in the first cycle were subsequently symptom free. However, for those women who continued to experience itching by the end of the trial, the mean duration increased from 1.4 to 7.3 (±11) days. The magnitude of the increase resulted from the fact that one patient reported itching for 20 days in the final study cycle.

Only one patient noted a problem with patch adhesion despite full normal activity that included bathing. Some patients commented that a "plaster rim" remained after the patch was removed. Patient acceptance of the Ortho-Cilag patch was high. All 20 patients found that it was comfortable and nonobstructive. It did not interfere with clothing or disturb the skin. Seventeen (85%) expressed willingness to continue use of this method of estrogen replacement.

Comment

Because of differences in study population and method, relief of symptoms from these two clinical trials cannot be directly compared. All of the women who used the Ciba-Geigy patch had already experienced relief of their symptoms from their previous estrogen replacement therapy, whereas in the Ortho-Cilag trial, the patients were either new to therapy or had not received treatment for at least 2 months and experienced symptoms at the start of the trial. This difference could partly account for the greater percentage of patients who wanted to continue use of the Ortho-Cilag patch (85% vs 25%). Another possible source of bias could be that the two study groups had different clinic motivators.

Adequate progestin therapy is necessary to prevent risk of endometrial cancer in women who undergo estrogen replacement therapy.[17] Progestins may, however, be associated with some adverse symptoms. Therefore our general policy is to gradually step up

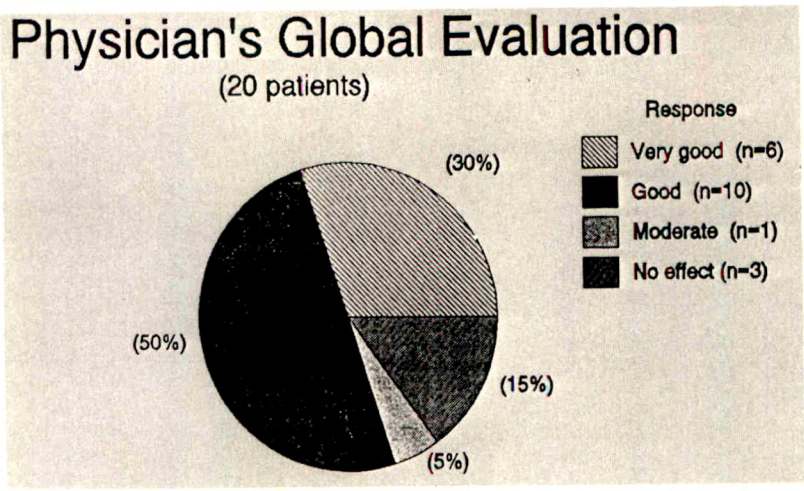


Fig. 4. Physician's global assessment of Ortho-Cilag transdermal estradiol patch after a 6-month trial.

the dosage until withdrawal bleeding occurs only at the end of the progestin exposure.[18] The starting dose of 0.35 mg/24 hr, as supplied for the trial, was too low and was increased in nine patients before the end of the 6-month study period.

We believe that certain conclusions can be drawn from the two studies with regard to adhesive properties of the patches and skin complications. Both products were designed to release 50 μg of estradiol per 24 hours. Furthermore, the racial distribution of the participants and the climate were the same in both studies. Although patients in the first trial were not specifically queried about patch adhesion, nine patients volunteered the fact that patches came off. In the second trial, however, patients were asked about patch adhesion, and only one patient acknowledged loss of patches in three of the six cycles.

The more severe reactions associated with the Ciba-Geigy patches appeared to be related to the combination of a tropical climate, an occlusive membrane, and the alcohol in the reservoir. Accumulation of sweat under patches has been associated with increased skin irritation from other transdermal systems,[19] and reports of unacceptable reactions to the Ciba-Geigy transdermal estradiol patch have also come from Thailand.[20, 21] The Ortho-Cilag patch also has an occlusive element but does not contain ethanol.

Reports of cutaneous reactions from the Ciba-Geigy patch in temperate climates range from none[22] to 35%.[23] Hogan and Maibach[24] cite a frequency of 17% with a 2% discontinuation rate. Holdiness[25] reviewed studies that reported a range of 0% to 36%. Higher rates have been reported in warmer areas of the United States, such as Florida.[26] McBurney et al.[27] reported nine patients in New Orleans who had dermatitis from the Ciba-Geigy patch, which they attributed to delayed contact sensitivity to substances in the system, including the adhesive (mineral oil and polyisobutylene) and the hydroxypropyl cellulose that was used as a gel in the reservoir. Their results were confusing, however, and they concluded that the reactions were to "either the adhesive or the vehicle" and further suggested that the drug reservoir might contain another allergen that caused contact sensitivity. Schwartz and Clendenning[28] reported a reaction to hydroxypropyl cellulose in alcohol and mineral oil but not in water.

The mitigating effect of estradiol observed in the patch component study is of particular interest. Utian[29] reported a much higher rate of cutaneous reactions with use of placebo patches versus estradiol patches. He did not mention, however, whether the placebo patches contained ethanol.

Prolonged pigmentation in two women that was still visible 4 months after removal of the Ciba-Geigy patch was a distressing adverse effect. Because estrogen is associated with increased pigmentation during pregnancy, it is possible that exposure to ultraviolet light and topical estradiol might increase local pigmentation. However, the Ciba-Geigy phototoxicity and photoallergy studies[9] had not demonstrated any potentiation. A recent report[30] of hyperpigmentation in a menopausal woman who was treated with ultraviolet phototherapy for pruritus that was caused by a transdermal estradiol system is of interest because of the analogy to the Singapore climate, although most of our patients would go out of their way to avoid unnecessary exposure to sunlight.

Conclusion

A trial that directly compares the Ortho-Cilag and the Ciba-Geigy 50 μg estradiol patches would provide more valid assessments of the relative efficacy, tolera-

bility, and adhesive properties of the two products. Nevertheless, some general observations can be made from this study. First, both patches are effective in relieving the symptoms of menopause, although they may be slightly less effective than the oral preparations in "equivalent"[31] doses. Second, the Ortho-Cilag patches are considerably less irritating to the skin when used by women who live in Singapore. Third, the Ortho-Cilag patches have better adhesive properties when used by women who live in Singapore. The last two advantages, combined with efficacy comparable with that of the Ciba-Geigy patches, make the Ortho-Cilag 50 μg estradiol transdermal patch more acceptable for use in tropical climates.

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Discussion II

Chairman Mishell. Is it acceptable to give hormone replacement therapy to women who have had endometrial or ovarian cancer more than 5 years earlier?

Dr. McCarthy. Yes. It certainly appears safe to give hormone replacement therapy to women after bilateral oophorectomy for epithelial ovarian cancer, even though there have been some suggestions that the ovary contains estrogen receptors. In a recent study[1] from the Royal Marsden Hospital in the United Kingdom, no significant difference in survival was found between users and nonusers of hormone replacement therapy that was begun at a median of 4 months after diagnosis.

It is somewhat more difficult to make a recommendation for women who have had endometrial cancer, but if the disease was at a very early stage at the time of operation,[2] I believe hormone replacement therapy can be given to such a patient who is clinically free of recurrence after 5 years.

Chairman Mishell. Dr. Lobo, if progestin is given to patients every 3 months instead of every month, will the effects of this steroid be reduced?

Dr. Lobo. I use it selectively. Those patients who cannot or will not take progestin must be monitored. I currently use ultrasound monitoring; when I see excessive proliferative effects, I prescribe progestin in an intermittent form. Although we do not yet have long-term data, I believe this is an appropriate procedure for managing those patients.

With the current medicolegal climate in the United States, an additional concern is that those who practice outside of what is normally recommended must take some extra precautions. These patients probably should have biopsies until we are absolutely certain ultrasonography is a safe method of monitoring. However, I am comfortable with the use of ultrasonography.

Chairman Mishell. Do you believe it is necessary to increase the dose of progestin when you give higher doses of estrogen to women who have severe hot flushes?

Dr. Lobo. The study by Gibbons et al.[3] showed that 2.5 mg of medroxyprogesterone acetate was protective, that it prevented the synthesis of the endometrial receptors for 0.625 mg of conjugated equine estrogens, and that 2.5 mg of medroxyprogesterone acetate was not sufficient for 1.25 mg of conjugated equine estrogen. A balance of estrogen and progestin is required. If a large amount of estrogen is used, the progestin must be increased; the size of the increase depends on how much the estrogen is increased.

Chairman Mishell. Dr. Harlap, why do epidemiologists use log scales rather than linear scales for setting the data? Doesn't this minimize the significance of the findings?

Dr. Harlap. It is mathematically more correct to use a log scale in presenting the data. By looking at the results plotted on a log scale, we obtain a "fairer" perception of what the differences mean. The confidence limits of the relative risk are also distributed in a log fashion. As Dr. Grimes pointed out, the relative risk of 0.5 is equal but opposite to a relative risk of 2. Similarly, a change in relative risk from 0.5 to 1 represents a doubling of risk, and a change from 1 to 2 represents a similar doubling.

Chairman Mishell. Dr. Lobo, what is your opinion of giving low-dose oral contraceptives as a means of estrogen replacement to women in developing countries in which estrogen replacement drugs are more expensive than oral contraceptives?

Dr. Lobo. Age is the deciding factor. If the patient has premature ovarian failure, I believe it is an acceptable practice. I would not give a low-dose oral contraceptive to a woman who is more than 60 years of age; I believe that is more estrogen than she needs. It is acceptable, however, for a nonsmoking woman to use these low-dose oral contraceptives up to or a little past the age of 45 years. However, if you look at dose equivalencies, even 30 μg of ethinyl estradiol in the pill is close to about 3 mg of conjugated equine estrogen, and that is much more than is really needed. As we have said several times the risk here is thrombosis and not atherosclerosis.

Dr. McCarthy. I would like to add a point regarding the price of estrogen replacement therapy, which must be balanced against the savings achieved when it is used in prophylactic treatment. Given that perspective, having a number of women who use estrogen will provide a savings to any society.

Chairman Mishell. Your data show that hormone replacement therapy saves lives, Dr. Harlap, but for how long, and with what quality of extended life?

Dr. Harlap. The question relates to the value of women to society. A woman aged 60 years or more whose health is preserved can be a valuable member of society both in developing and developed countries. That it is beneficial to postpone death and to increase productive or valuable life is without question.

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Dr. Lobo. The leading cause of death in women is still cardiovascular disease, but with replacement therapy life can be prolonged for 2½ to 3 years.

Chairman Mishell. Dr. Lobo, no evidence exists that progestin prevents breast cancer. Would you suggest giving it to patients who have had a hysterectomy?

Dr. Lobo. We do not advocate giving progestin to patients who have had a hysterectomy. We do not believe that progestin has a protective effect against breast cancer.

Chairman Mishell. Dr. Lindsay, in what order would you rank the following factors in preventing osteoporosis: exercise, cessation of smoking, a calcium-rich diet (calcium supplementation), and estrogen replacement therapy?

Dr. Lindsay. For postmenopausal women the only effective agent among those listed is estrogen replacement therapy. The evidence is clear that calcium supplementation and exercise do not alone prevent the bone loss caused by ovarian insufficiency. Nonetheless, these factors improve general health and thus have beneficial effects beyond the effects on the skeleton. In treating patients the physician should recommend a reasonable program of physical activity, discourage smoking, and ensure that calcium deficiency does not exacerbate the effects of estrogen deprivation. Patients should obtain between 1000 and 1500 mg of calcium per day, preferably from dietary sources.

Chairman Mishell. Would steroid treatment benefit a woman who is already 10 years postmenopausal and has not previously received this treatment?

Dr. Lindsay. There appears to be some doubt in the literature, or at least in clinicians' minds, about the value of estrogen intervention in older patients or in patients who already have clinical osteoporosis. However, several studies[4] have demonstrated that replacement therapy protects women up to age 75 years and those with established osteoporosis against further bone loss. Estrogen intervention is the first-line therapy for osteoporosis in women who are at least 75 years of age.

Chairman Mishell. Dr. McCarthy, could you comment on the advantages or disadvantages of transdermal estrogen versus oral estrogen?

Dr. McCarthy. I made two general points about the absence of the first pass to the liver and the fact that a constant level in the blood is present rather than an up-and-down effect. I believe both those effects are well established. The advantages and relative advantages and disadvantages are being assessed.

Chairman Mishell. Dr. McCarthy, you discussed the regulation of estrogens by membrane. In the matrix patch the skin is the only straight filter. Were any differences in plasma estradiol levels obtained with the two patch systems?

Dr. McCarthy. Ours was not a direct comparison between the two transdermal systems. After our adverse experience with the initial patch we thought that many patients might withdraw in the middle of the trial; therefore we studied only 20 patients. We were mainly interested in the acceptability and did not measure serum estradiol levels in the second series.

Chairman Mishell. Another question that arose is: Why not give estrogen to men because of its protective effects against coronary heart disease? I believe the answer is that it stimulates breast growth and increases the risk of breast cancer in men. I believe other agents exist that can affect the lipids beneficially.

Another question that arose is whether estrogen replacement can be given routinely to all women on a prophylactic basis; I believe this can be done.

Participant. I agree. We basically have full proof of the protective effect; a recent article in the *New England Journal of Medicine* (1991;325:756-62) confirms that proof.

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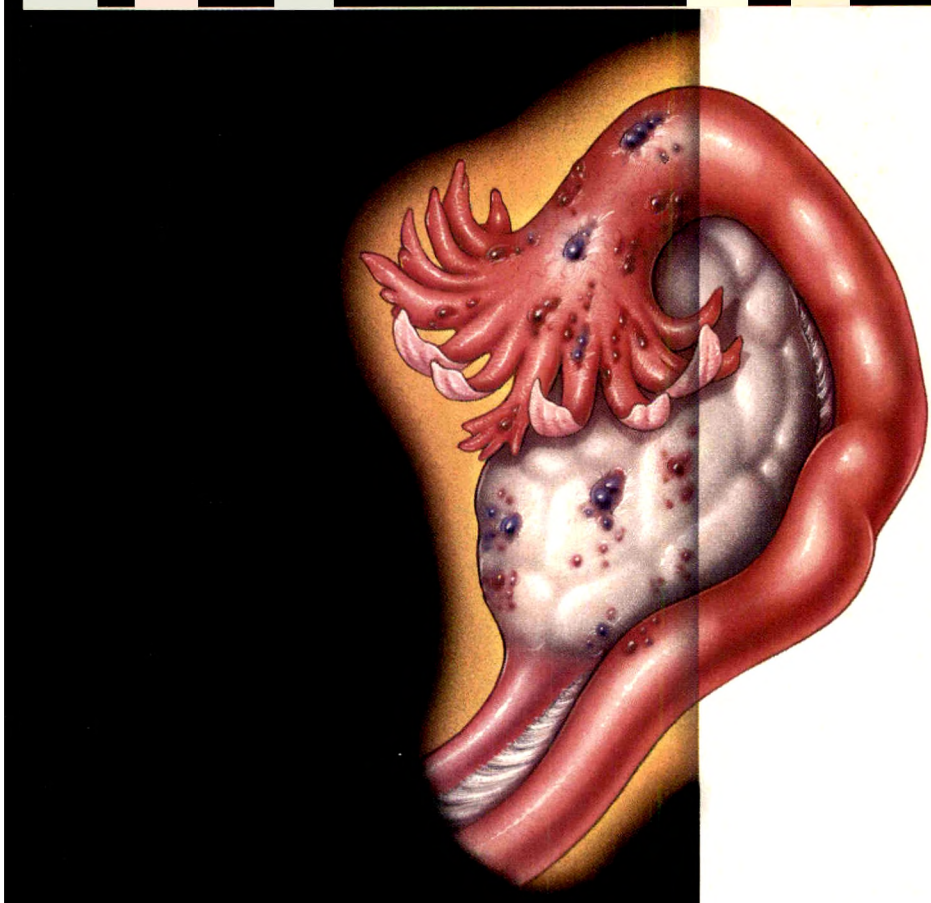


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BRIEF SUMMARY

SYNAREL® Nasal Solution contains nafarelin acetate (2 mg/mL, expressed as nafarelin base), benzalkonium chloride, glacial acetic acid, sodium hydroxide or hydrochloric acid, sorbitol, and purified water.

INDICATIONS AND USAGE: For management of endometriosis. Experience is limited to women 18 years and older treated for 6 months.

CONTRAINDICATIONS: Hypersensitivity to GnRH, GnRH agonist analogs or any excipients in SYNAREL; undiagnosed abnormal vaginal bleeding; pregnancy or possibility of pregnancy while taking the drug because SYNAREL may cause fetal harm when administered to a pregnant woman. If SYNAREL is used during pregnancy or if the patient becomes pregnant while taking this drug, she should be apprised of the potential hazard to the fetus; use in women who are breast feeding.

WARNINGS: Safe use in pregnancy has not been established clinically. Before starting treatment pregnancy must be excluded.

When used regularly at the recommended dose, SYNAREL usually inhibits ovulation and stops menstruation. Contraception is not insured, particularly if patients miss successive doses. Therefore, patients should use nonhormonal contraception. Advise patients to see their physician if they believe they may be pregnant. If a patient becomes pregnant during treatment, discontinue the drug; apprise patient of the potential risk to the fetus.

PRECAUTIONS General: Ovarian cysts have been reported in the first 2 months of therapy. These may resolve spontaneously or may require drug discontinuation or surgery.

Information for Patients: 1. Since menstruation should stop with SYNAREL, the patient should notify her physician if regular menstruation persists. If patient is compliant and menstruation persists to second month, consider doubling the dose. Counsel noncompliant patients on importance of taking SYNAREL regularly. 2. Patients should not use SYNAREL if pregnant, breast feeding, have undiagnosed abnormal vaginal bleeding, or are allergic to any of its ingredients. 3. Safe use in pregnancy has not been established. Therefore, a nonhormonal method of contraception should be used during treatment. If a patient becomes pregnant during treatment, she should discontinue the drug and consult her physician. 4. Adverse events occurring most frequently in clinical studies are associated with hypoestrogenism. Nasal irritation occurred in about 10% of patients. 5. Induced hypoestrogenic state results in a small loss in bone density over a 6-month treatment course. Some of this loss may not be reversible. During one six-month treatment period, this should not be important. In patients with major risk factors for decreased bone mineral content, i.e., chronic alcohol and/or tobacco use, strong family history of osteoporosis, or chronic use of drugs that can reduce bone mass such as anticonvulsants or corticosteroids, SYNAREL may pose additional risk. In these patients risks and benefits must be weighed carefully. Repeated courses of treatment with GnRH analogs are not advisable in patients with major risk factors for loss of bone mineral content. 6. Patients with intercurrent rhinitis should consult their physician for use of a topical nasal decongestant. If a topical nasal decongestant is required during treatment, it must be used at least 30 minutes after SYNAREL dosing to decrease the possibility of reducing drug absorption. 7. Retreatment cannot be recommended since safety data beyond 6 months are not available.

Drug Interactions: No pharmacokinetic-based drug-drug interaction studies have been conducted. Nafarelin acetate is a peptide primarily degraded by peptidase and not by cytochrome P-450 enzymes, and is only about 80% bound to plasma proteins at 4°C; therefore drug interactions would not be expected to occur.

Drug/Laboratory Test Interactions: SYNAREL in therapeutic doses suppresses the pituitary-gonadal system. Normal function is usually restored 4-8 weeks after treatment is discontinued. Therefore, diagnostic tests of pituitary gonadotropic and gonadal functions during treatment and up to 4-8 weeks after discontinuation of SYNAREL may be misleading.

Carcinogenesis, Mutagenesis, Impairment of Fertility: Carcinogenicity studies were conducted in rats and mice at intramuscular doses up to 110 times and 560 times the maximum recommended human intranasal dose, respectively. These multiples of the human dose are based on the relative bioavailability of the drug by the two routes of administration. As with other GnRH agonists, nafarelin acetate given to laboratory rodents at high doses for prolonged periods induced hyperplasia and/or neoplasia of endocrine organs. At 24 months, there was an increase in the incidence of pituitary tumors (adenoma/carcinoma) in high-dose female rats and a dose-related increase in male rats. There was an increase in pancreatic islet cell adenomas in both sexes, and in benign testicular and ovarian tumors in the treated groups. There was a dose-related increase in benign adrenal medullary tumors in treated female rats. In mice, there was a dose-related increase in Harderian gland tumors in males and an increase in pituitary adenomas in high-dose females. No metastases of these tumors were observed. It is known that tumorigenicity in rodents is particularly sensitive to hormonal stimulation.

Mutagenicity studies using bacterial, yeast, and mammalian systems provided no evidence of mutagenic potential.

Reproduction studies in male and female rats have shown full reversibility of fertility suppression when treatment was discontinued after continuous administration for up to 6 months.

Pregnancy, Teratogenic Effects: *Pregnancy Category X.* Intramuscular SYNAREL was administered to rats throughout gestation at about 0.5, 2, and 7 times the maximum recommended human intranasal dose based on the relative bioavailability by the two routes of administration. An increase in major fetal abnormalities was seen in 4/80 fetuses at the highest dose. A similar study at the same doses in rats and studies in mice and rabbits at doses up to 600 μg/kg/day and 0.18 μg/kg/day, respectively, failed to demonstrate an increase in fetal abnormalities after administration throughout gestation. In rats and rabbits, there was a dose-related increase in fetal mortality and a decrease in fetal weight with the highest dose.

Nursing Mothers: It is not known whether SYNAREL is excreted in human milk. Because many drugs are excreted in human milk, and because the effects of SYNAREL on lactation and/or the breastfed child have not been determined, SYNAREL should not be used by nursing mothers.

Pediatric Use: Safety and effectiveness in children have not been established.

ADVERSE REACTIONS: The most frequently reported adverse reactions were those related to hypoestrogenism. In controlled studies comparing SYNAREL (400 μg/day) and danazol (600 or 800 mg/day), the following adverse reactions were most frequently reported and thought to be drug-related.

Hypoestrogenic: hot flashes (SYNAREL 90%, danazol 69%), decreased libido (22%, 7%), vaginal dryness (19%, 7%), headaches (19%, 21%), emotional lability (15%, 18%), insomnia (8%, 4%); **Androgenic:** acne (13%, 20%), myalgia (10%, 23%), reduced breast size (10%, 16%), edema (8%, 23%), seborrhea (8%, 17%), weight gain (8%, 28%), hirsutism (2%, 6%), increased libido (1%, 6%); **Local:** nasal irritation (10%, 3%); **Miscellaneous:** depression (2%, 5%), weight loss (1%, 3%).

Less than 1% of patients experienced paresthesia, palpitations, chloasma, maculopapular rash, eye pain, urticaria, asthenia, lactation, breast engorgement, and arthralgia. In clinical trials, immediate hypersensitivity thought to be possibly or probably related to nafarelin occurred in 3 (0.2%) of 1509 healthy subjects or patients.

Changes in Bone Density: After 6 months' treatment vertebral trabecular bone density and total vertebral bone mass, measured by quantitative computed tomography (QCT), decreased by an average of 8.7% and 4.3%, respectively, compared to pretreatment levels. There was partial recovery of bone density post-treatment; the average trabecular bone density and total bone mass were 4.9% and 3.3% less than pretreatment levels, respectively. Total vertebral bone mass, measured by dual photon absorptiometry (DPA), decreased by a mean of 5.9% at end of treatment. Mean total vertebral mass, re-examined by DPA 6 months post-treatment, was 1.4% below pretreatment levels. There was little, if any, decrease in the mineral content in compact bone of the distal radius and second metacarpal. Use for longer than the recommended 6 months or in presence of other known risk factors for decreased bone mineral content may cause additional bone loss.

Changes in Laboratory Values: *Plasma enzymes.* During clinical trials SGOT and SGPT levels were more than twice the upper limit of normal in only one patient each. There was no other evidence of abnormal liver function and levels returned to normal after treatment was stopped.

Lipids. At enrollment and at end of treatment, 9% of patients on SYNAREL 400 μg/day and 2% on danazol had total cholesterol values above 250 mg/dL.

Of those with pretreatment cholesterol values below 250 mg/dL, 6% on SYNAREL and 18% on danazol had post-treatment values above 250 mg/dL.

Mean (±SEM) pretreatment values for total cholesterol from all patients were 191.8 (4.3) mg/dL in the SYNAREL group and 193.1 (4.6) mg/dL in the danazol group. At the end of treatment, mean values from all patients were 204.5 (4.8) mg/dL in the SYNAREL group and 207.7 (5.1) mg/dL in the danazol group. These increases from pretreatment values were significant ($p < 0.05$) in both groups.

Triglycerides were increased above the upper limit of 150 mg/dL in 12% of the patients on SYNAREL and in 7% of the patients on danazol.

At the end of treatment, no patients on SYNAREL had abnormally low HDL cholesterol fractions (< 30 mg/dL) compared with 4.3% of patients on danazol. No patients on SYNAREL had abnormally high LDL cholesterol fractions (> 190 mg/dL) compared with 15% of those on danazol. There was no increase in the LDL/HDL ratio in patients on SYNAREL, but approximately a 2-fold increase in the LDL/HDL ratio in patients on danazol.

Other changes. In comparative studies, the following changes were seen in approximately 10% to 15% of patients. SYNAREL was associated with elevations of plasma phosphorous and eosinophil counts, and decreases in serum calcium and WBC counts. Danazol therapy was associated with an increase of hematocrit and WBC.

OVERDOSAGE: At present there is no clinical evidence of adverse effects following overdosage of GnRH analogs.

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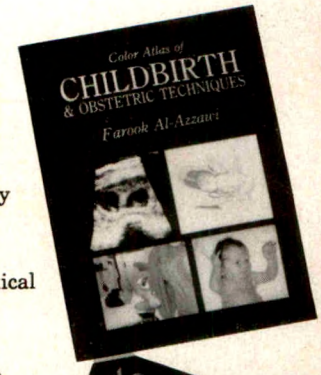
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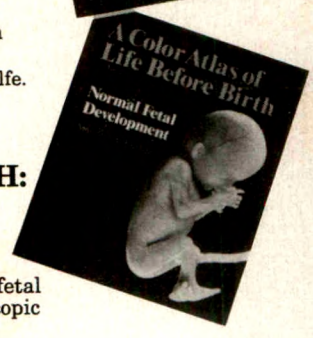


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CLINICAL SECTION

Clinical Articles

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T.C. Hillard, MD, N.C. Siddle, MD, M.I. Whitehead, MD, D.I. Fraser, MD, and J. Pryse-Davies, MD

London, England

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Raphael N. Pollack, MDCM, Gittit Hauer-Pollack, MSc, and Michael Y. Divon, MD

Bronx, New York

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James A. Low, MD, Lynn L. Simpson, MD, and David A. Ramsey, MB, ChB

Kingston, Ontario, Canada

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Milwaukee, Wisconsin, and Chicago, Illinois

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Laurence B. McCullough, PhD, John Coverdale, MB, ChB, Timothy Bayer, MD, and Frank A. Chervenak, MD

Houston, Texas, and New York, New York

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Henk J. Out, MD, Hein W. Bruinse, MD, Godelieve C.M.L. Christiaens, MD, Marja van Vliet, Philip G. de Groot, PhD, H. Karel Nieuwenhuis, MD, and Ronald H.W.M. Derksen, MD

Utrecht, The Netherlands

In a multicenter controlled study it is demonstrated prospectively that the presence of antiphospholipid antibodies is a risk factor for adverse pregnancy outcome.

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INDICATIONS AND USAGE
TREATMENT

CEFOTAN is indicated for the therapeutic treatment of the following infections when caused by susceptible strains of the designated organisms:

Urinary Tract Infections caused by *E. coli, Klebsiella* species (including *K. pneumoniae*), *Proteus mirabilis,* and *Proteus* sp (which may include the organisms now called *Proteus vulgaris, Providencia rettgeri,* and *Morganella morganii*).

Lower Respiratory Tract Infections caused by *Streptococcus pneumoniae* (formerly *D. pneumoniae*), *Staphylococcus aureus* (penicillinase- and nonpenicillinase-producing strains), *Haemophilus influenzae* (including ampicillin-resistant strains), *Klebsiella* species (including *K. pneumoniae*), *E. coli, Proteus mirabilis,* and *Serratia marcescens**.

Skin and Skin Structure Infections due to *Staphylococcus aureus* (penicillinase- and nonpenicillinase-producing strains), *Staphylococcus epidermidis, Streptococcus pyogenes, Streptococcus* species (excluding enterococci), *Escherichia coli, Klebsiella pneumoniae, Peptococcus niger**, *Peptostreptococcus* species.

Gynecologic Infections caused by *Staphylococcus aureus* (including penicillinase- and nonpenicillinase-producing strains), *Staphylococcus epidermidis, Streptococcus* species (excluding enterococci), *Streptococcus agalactiae, E. coli, Proteus mirabilis, Neisseria gonorrhoeae,* Bacteroides species (excluding *B. distasonis, B. ovatus, B. thetaiotaomicron*), *Fusobacterium* species*, and gram-positive anaerobic cocci (including *Peptococcus niger* and *Peptostreptococcus* species).

Cefotetan, like other cephalosporins, has no activity against *Chlamydia trachomatis.* Therefore, when cephalosporins are used in the treatment of pelvic inflammatory disease, and *C. trachomatis* is one of the suspected pathogens, appropriate antichlamydial coverage should be added.

Intra-abdominal Infections caused by *E. coli, Klebsiella* species (including *K. pneumoniae*), *Streptococcus* species (excluding enterococci), *Bacteroides* species (excluding *B. distasonis, B. ovatus, B. thetaiotaomicron*) and *Clostridium* species*.

Bone and Joint Infections caused by *Staphylococcus aureus**.

*Efficacy for this organism in this organ system was studied in fewer than ten infections.

Specimens for bacteriological examination should be obtained in order to isolate and identify causative organisms and to determine their susceptibilities to cefotetan. Therapy may be instituted before results of susceptibility studies are known; however, once these results become available, the antibiotic treatment should be adjusted accordingly.

In cases of confirmed or suspected gram-positive or gram-negative sepsis or in patients with other serious infections in which the causative organism has not been identified, it is possible to use CEFOTAN concomitantly with an aminoglycoside. Cefotetan combinations with aminoglycosides have been shown to be synergistic *in vitro* against many Enterobacteriaceae and also some other gram-negative bacteria. The dosage recommended in the labeling of both antibiotics may be given and depends on the severity of the infection and the patient's condition.

NOTE: If CEFOTAN and an aminoglycoside are used concomitantly, renal function should be carefully monitored, especially if higher dosages of the aminoglycoside are to be administered or if therapy is prolonged, because of the potential nephrotoxicity and ototoxicity of aminoglycosidic antibiotics. Although, to date, nephrotoxicity has not been noted when CEFOTAN was given alone, it is possible that nephrotoxicity may be potentiated if CEFOTAN is used concomitantly with an aminoglycoside.

PROPHYLAXIS

The preoperative administration of CEFOTAN may reduce the incidence of certain postoperative infections in patients undergoing surgical procedures that are classified as clean contaminated or potentially contaminated (e.g., cesarean section, abdominal or vaginal hysterectomy, transurethral surgery, biliary tract surgery, and gastrointestinal surgery).

The prophylactic dose of CEFOTAN should be administered 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, CEFOTAN should be administered intravenously after the clamping of the umbilical cord.

If there are signs and symptoms of infection, specimens for culture should be obtained for identification of the causative organism so that appropriate therapeutic measures may be initiated.

CONTRAINDICATIONS

CEFOTAN is contraindicated in patients with known allergy to the cephalosporin group of antibiotics.

WARNINGS

BEFORE THERAPY WITH CEFOTAN IS INSTITUTED, CAREFUL INQUIRY SHOULD BE MADE TO DETERMINE WHETHER THE PATIENT HAS HAD PREVIOUS HYPERSENSITIVITY REACTIONS TO CEFOTETAN DISODIUM, CEPHALOSPORINS, PENICILLINS, OR OTHER DRUGS. IF THIS PRODUCT IS TO BE GIVEN TO PENICILLIN-SENSITIVE PATIENTS, CAUTION SHOULD BE EXERCISED BECAUSE CROSS-HYPERSENSITIVITY AMONG BETA-LACTAM ANTIBIOTICS HAS BEEN CLEARLY DOCUMENTED AND MAY OCCUR IN UP TO 10% OF PATIENTS WITH A HISTORY OF PENICILLIN ALLERGY. IF AN ALLERGIC REACTION TO CEFOTAN OCCURS, DISCONTINUE THE DRUG. SERIOUS ACUTE HYPERSENSITIVITY REACTIONS MAY REQUIRE TREATMENT WITH EPINEPHRINE AND OTHER EMERGENCY MEASURES, INCLUDING OXYGEN, INTRAVENOUS FLUIDS, INTRAVENOUS ANTIHISTAMINES, CORTICOSTEROIDS, PRESSOR AMINES AND AIRWAY MANAGEMENT, AS CLINICALLY INDICATED.

Pseudomembranous colitis has been reported with nearly all antibacterial agents, including cefotetan, and may range from mild to life-threatening. Onset of pseudomembranous colitis symptoms may occur during or after antibiotic treatment or surgical prophylaxis. Therefore, it is important to consider this diagnosis in patients who present with diarrhea subsequent to the administration of antibacterial agents.

Treatment with antibacterial agents alters the normal flora of the colon and may permit overgrowth of clostridia. Studies indicate that a toxin produced by *Clostridium difficile* is a primary cause of "antibiotic-associated colitis."

After the diagnosis of pseudomembranous colitis has been established, therapeutic measures should be initiated. Mild cases of pseudomembranous colitis usually respond to discontinuation of the drug alone. In moderate to severe cases, consideration should be given to management with fluids and electrolytes, protein supplementation, and treatment with an oral antibacterial drug effective against *Clostridium difficile.*

In common with many other broad-spectrum antibiotics, CEFOTAN may be associated with a fall in prothrombin activity and, possibly, subsequent bleeding. Those at increased risk include patients with renal or hepatobiliary impairment or poor nutritional state, the elderly, and patients with cancer. Prothrombin time should be monitored and exogenous vitamin K administered as indicated.

PRECAUTIONS

General: As with other broad-spectrum antibiotics, prolonged use of CEFOTAN may result in overgrowth of nonsusceptible organisms. Careful observation of the patient is essential. If superinfection does occur during therapy, appropriate measures should be taken.

CEFOTAN should be used with caution in individuals with a history of gastrointestinal disease, particularly colitis.

Information for Patients: As with some other cephalosporins, a disulfiram-like reaction characterized by flushing, sweating, headache, and tachycardia may occur when alcohol (beer, wine, etc.) is ingested within 72 hours after CEFOTAN administration. Patients should be cautioned about the ingestion of alcoholic beverages following the administration of CEFOTAN.

Drug Interactions: Although to date nephrotoxicity has not been noted when CEFOTAN was given alone, it is possible that nephrotoxicity may be potentiated if CEFOTAN is used concomitantly with an aminoglycoside.

Drug/Laboratory Test Interactions: The administration of CEFOTAN may result in a false-positive reaction for glucose in the urine using Clinitest®†, Benedict's solution, or Fehling's solution. It is recommended that glucose tests based on enzymatic glucose oxidase be used.

As with other cephalosporins, high concentrations of cefotetan may interfere with measurement of serum and urine creatinine levels by Jaffe reaction and produce false increases in the levels of creatinine reported.

Carcinogenesis, Mutagenesis, Impairment of Fertility: Although long-term studies in animals have not been performed to evaluate carcinogenic potential, no mutagenic potential of cefotetan was found in standard laboratory tests.

Cefotetan has adverse effects on the testes of prepubertal rats. Subcutaneous administration of 500 mg/kg/day (approximately 8-16 times the usual adult human dose) on days 6-35 of life (thought to be developmentally analogous to late childhood and prepuberty in humans) resulted in reduced testicular weight and seminiferous tubule degeneration in 10 of 10 animals. Affected cells included spermatogonia and spermatocytes; Sertoli and Leydig cells were unaffected. Incidence and severity of lesions were dose-dependent; at 120 mg/kg/day (approximately 2-4 times the usual human dose) only 1 of 10 treated animals was affected, and the degree of degeneration was mild.

Similar lesions have been observed in experiments of comparable design with other methylthiotetrazole-containing antibiotics and impaired fertility has been reported, particularly at high dose levels. No testicular effects were observed in 7-week-old rats treated with up to 1000 mg/kg/day SC for 5 weeks, or in infant dogs (3 weeks old) that received up to 300 mg/kg/day IV for 5 weeks. The relevance of these findings to humans is unknown.

Pregnancy: Teratogenic Effects. Pregnancy Category B: Reproduction studies have been performed in rats and monkeys at doses up to 20 times the human dose and have revealed no evidence of impaired fertility or harm to the fetus due to cefotetan. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproductive studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Nursing Mothers: Cefotetan is excreted in human milk in very low concentrations. Caution should be exercised when cefotetan is administered to a nursing woman.

Pediatric Use: Safety and effectiveness in children have not been established.

ADVERSE REACTIONS

In clinical studies, the following adverse effects were considered related to CEFOTAN therapy. Those appearing in italics have been reported during postmarketing experience.

Gastrointestinal symptoms occurred in 1.5% of patients; the most frequent were diarrhea (1 in 80) and nausea (1 in 700); *pseudomembranous colitis.* Onset of pseudomembranous colitis symptoms may occur during or after antibiotic treatment or surgical prophylaxis. (See WARNINGS.)

Hematologic laboratory abnormalities occurred in 1.4% of patients and included eosinophilia (1 in 200), positive direct Coombs' test (1 in 250), and thrombocytosis (1 in 300); *agranulocytosis, hemolytic anemia, leukopenia, thrombocytopenia,* and *prolonged prothrombin time.*

Hepatic enzyme elevations occurred in 1.2% of patients and included a rise in SGPT (1 in 150), SGOT (1 in 300), alkaline phosphatase (1 in 700), and LDH (1 in 700).

Hypersensitivity reactions were reported in 1.2% of patients and included rash (1 in 150) and itching (1 in 700); *anaphylactic reactions.*

Local effects were reported in less than 1.0% of patients and included phlebitis at the site of injection (1 in 300), and discomfort (1 in 500).

Miscellaneous: *Fever*

In addition to the adverse reactions listed above which have been observed in patients treated with cefotetan, the following adverse reactions and altered laboratory tests have been reported for cephalosporin-class antibiotics: urticaria, pruritis, Stevens-Johnson syndrome, erythema multiforme, toxic epidermal necrolysis, vomiting, abdominal pain, colitis, superinfection, vaginitis including vaginal candidiasis, renal dysfunction, toxic nephropathy, hepatic dysfunction including cholestasis, aplastic anemia, hemorrhage, increased BUN, increased creatinine, elevated bilirubin, pancytopenia, and neutropenia.

Several cephalosporins have been implicated in triggering seizures, particularly in patients with renal impairment when the dosage was not reduced (see DOSAGE AND ADMINISTRATION and OVERDOSAGE). If seizures associated with drug therapy occur, the drug should be discontinued. Anticonvulsant therapy can be given if clinically indicated.

DOSAGE AND ADMINISTRATION
TREATMENT

The usual adult dosage is 1 or 2 grams of CEFOTAN administered intravenously or intramuscularly every 12 hours for 5 to 10 days. Proper dosage and route of administration should be determined by the condition of the patient, severity of the infection, and susceptibility of the causative organism.

GENERAL GUIDELINES FOR DOSAGE OF CEFOTAN

Type of Infection	Daily Dose	Frequency and Route
Urinary Tract	1-4 grams	500 mg every 12 hours IV or IM 1 or 2 g every 24 hours IV or IM 1 or 2 g every 12 hours IV or IM
Skin & Skin Structure		
Mild-Moderate[a]	2 grams	2 g every 24 hours IV 1 g every 12 hours IV or IM
Severe	4 grams	2 g every 12 hours IV
Other Sites	2-4 grams	1 or 2 g every 12 hours IV or IM
Severe	4 grams	2 g every 12 hours IV
Life-Threatening	6 grams[b]	3 g every 12 hours IV

[a] *K. pneumoniae* skin and skin structure infections should be treated with 1 or 2 grams every 12 hours IV or IM.

[b] Maximum daily dosage should not exceed 6 grams.

If *C. trachomatis* is a suspected pathogen in gynecologic infections, appropriate antichlamydial coverage should be added, since cefotetan has no activity against this organism.

PROPHYLAXIS

To prevent postoperative infection in clean contaminated or potentially contaminated surgery in adults, the recommended dosage is 1 or 2 g of CEFOTAN administered once, intravenously, 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, the dose should be administered as soon as the umbilical cord is clamped.

IMPAIRED RENAL FUNCTION

When renal function is impaired, a reduced dosage schedule must be employed. See FULL PRESCRIBING INFORMATION for a complete description.

NOTE: Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration whenever solution and container permit.

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References: 1. Walker CK, Landers DV, Ohm-Smith MJ, et al. Comparison of cefotetan plus doxycycline with cefoxitin plus doxycycline in the inpatient treatment of acute salpingitis. *Sex Trans Dis.* 1991;18:119-123. **2.** McGregor JA, Gordon SF, Krotec J, Poindexter AN. Results of a randomized, multicenter, comparative trial of a single dose of cefotetan versus multiple doses of cefoxitin as prophylaxis in cesarean section. *Am J Obstet Gynecol.* 1988;158:701-706. **3.** Carver P, Quintiliani R, Nightingale CH. Comparative pharmacokinetic study of cefotetan and cefoxitin in healthy volunteers. *Infect Surg.* April 1986(suppl):11-14. **4.** Ohm-Smith MJ, Sweet RL, Hadley WK. In vitro activity of cefbuperazone and other antimicrobial agents against isolates from the female genital tract. *Antimicrob Agents Chemother.* 1985;27:958-960. **5.** Data on file, Stuart Pharmaceuticals, Wilmington, Delaware. **6.** Souza Dias MB, Jacobus NV, Tally FP, Gorbach SL. Activity of cefotetan against anaerobic bacteria. *Diagn Microbiol Infect Dis.* 1986;4:359-363. **7.** Wexler HM, Finegold SM. In vitro activity of cefotetan compared with that of other antimicrobial agents against anaerobic bacteria. *Antimicrob Agents Chemother.* 1988;32:601-604. **8.** Hennessey TD. Cefotetan: a compilation of susceptibility data of clinical isolates. In: Lode H, Periti P, Strachan CJL, eds. *Cefotetan: A Long-Acting Antibiotic for Polymicrobial Infections.* New York, NY: Churchill Livingstone; 1985:14-22. **9.** Centers for Disease Control. 1989 sexually transmitted diseases treatment guidelines. *MMWR.* 1989;38(suppl S-8):1-43.

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Chicago, Illinois

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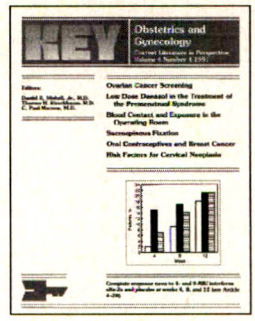
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Lexington, Kentucky, and Memphis, Tennessee

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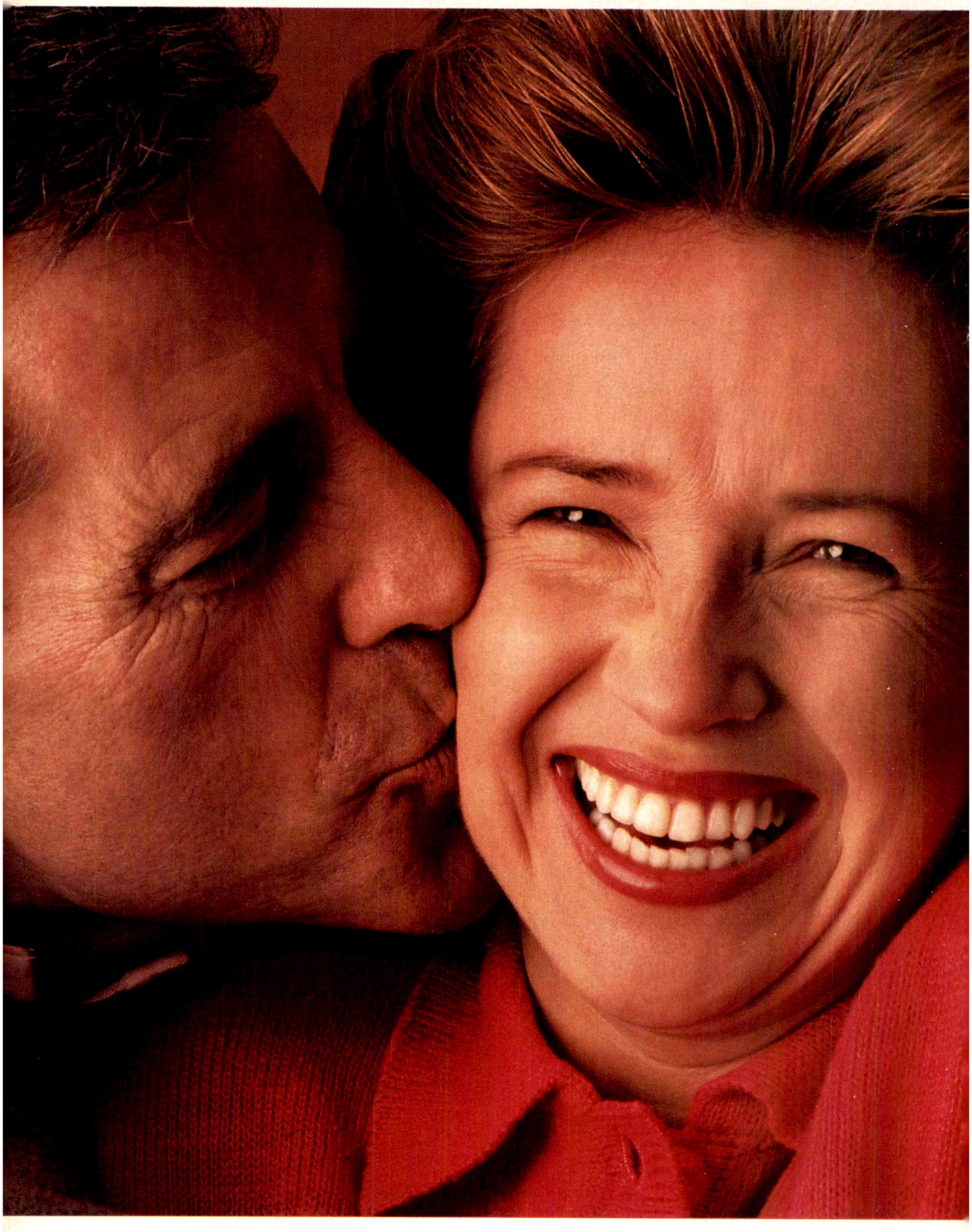
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PREMARIN® Brand of conjugated estrogens Vaginal Cream, in a nonliquefying base

1. ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA. Close clinical surveillance of all women taking estrogens is important. Adequate diagnostic measures including endometrial sampling when indicated should be undertaken to rule out malignancy in all cases of undiagnosed persistent or recurring abnormal vaginal bleeding. There is currently no evidence that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.
2. ESTROGENS SHOULD NOT BE USED DURING PREGNANCY.
Estrogen therapy during pregnancy is associated with an increased risk of congenital defects in the reproductive organs of the male and female fetus, an increased risk of vaginal adenosis, squamous-cell dysplasia of the uterine cervix, and vaginal cancer in the female later in life. The 1985 DES Task Force concluded that women who used DES during their pregnancies may subsequently experience an increased risk of breast cancer. However, a causal relationship is still unproven, and the observed level of risk is similar to that for a number of other breast cancer risk factors.
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DESCRIPTION: PREMARIN (conjugated estrogens, USP) contains a mixture of estrogens, obtained exclusively from natural sources, blended to represent the average composition of material derived from pregnant mares' urine. It contains estrone, equilin, and 17α-dihydroequilin, together with smaller amounts of 17α-estradiol, equilenin, and 17α-dihydroequilenin as salts of their sulfate esters. Tablets are available in 0.3 mg, 0.625 mg, 0.9 mg, 1.25 mg, and 2.5 mg strengths of conjugated estrogens. Cream is available as 0.625 mg conjugated estrogens per gram.

INDICATIONS AND USAGE: Moderate-to-severe vasomotor symptoms associated with the menopause. (There is no evidence that estrogens are effective for nervous symptoms or depression which might occur during menopause and they should not be used to treat these conditions.) Prevention and management of osteoporosis (abnormally low bone mass). Atrophic vaginitis. Atrophic urethritis. Hypoestrogenism due to hypogonadism, castration or primary ovarian failure.

PREMARIN (conjugated estrogens) Vaginal Cream is indicated in the treatment of atrophic vaginitis and kraurosis vulvae.

PREMARIN HAS NOT BEEN SHOWN TO BE EFFECTIVE FOR ANY PURPOSE DURING PREGNANCY AND ITS USE MAY CAUSE SEVERE HARM TO THE FETUS (SEE BOXED WARNING).

CONTRAINDICATIONS: Estrogens should not be used in women (or men) with any of the following conditions: 1. Known or suspected pregnancy (see Boxed Warning). 2. Known or suspected cancer of the breast except in appropriately selected patients being treated for metastatic disease. 3. Known or suspected estrogen-dependent neoplasia. 4. Undiagnosed abnormal genital bleeding. 5. Active thrombophlebitis or thromboembolic disorders. 6. Estrogen replacement therapy has not been reported to increase the risk of thrombophlebitis and/or thromboembolic disease. However, there is insufficient information regarding women who have had previous thromboembolic disease.

PREMARIN Tablets and Vaginal Cream should not be used in patients hypersensitive to their ingredients.

WARNINGS: Some studies suggest a possible increased incidence of breast cancer in women taking higher doses of estrogen for prolonged time periods. The majority of studies have not shown an association with usual estrogen replacement doses. Endometrial cancer risk among estrogen users was about 4-fold or greater than in non-users, and appears dependent on treatment duration and estrogen dose. In patients on combined estrogen-progestin therapy, this risk appears to be decreased. (See PRECAUTIONS below.)

Estrogen therapy during pregnancy is associated with an increased risk of fetal congenital reproductive tract disorders.

A 2.5-fold increase in the risk of surgically confirmed gall bladder disease in women receiving postmenopausal estrogens has been reported.

Large doses of estrogen such as those used to treat prostate and breast cancer have been shown to increase the risk of non-fatal myocardial infarction, pulmonary embolism, and thrombophlebitis in men. This cannot necessarily be extrapolated to women. However, to avoid theoretical cardiovascular risk caused by high estrogen doses, the doses for estrogen replacement therapy should not exceed the recommended dose.

Blood pressure should be monitored with estrogen use, especially if high doses are used.

Estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases.

PRECAUTIONS: The addition of a progestin for 7 or more days of a cycle of estrogen administration reportedly lowers the incidence of endometrial hyperplasia. Studies of endometrium suggest that 10 to 13 days of progestin are needed to provide maximal endometrial maturation and elimination of hyperplastic changes. Additional risks, such as adverse effects on carbohydrate and lipid metabolism, may be associated with the inclusion of progestin in estrogen replacement regimens. The choice of progestin and dosage may be important in minimizing these adverse effects.

Physical examination and a complete medical and family history should be taken prior to the initiation of any estrogen therapy with special reference to blood pressure, breasts, abdomen, and pelvic organs, and should include a Papanicolaou smear. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination being performed. Conditions influenced by fluid retention, such as asthma, epilepsy, migraine, and cardiac or renal dysfunction, require careful observation. Certain patients may develop manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding and mastodynia. Pre-existing uterine leiomyomata may increase in size during estrogen use. Estrogens should be used with care in patients with impaired liver function, renal insufficiency, or metabolic bone diseases associated with hypercalcemia.

The following drug/laboratory test interactions have been reported, some only with estrogen-progestin combinations (oral contraceptives):

1. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.
2. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by T_4 levels determined by column or by radioimmunoassay. Free T_3 resin uptake is decreased, reflecting the elevated TBG; free T_4 concentration is unaltered.
3. Impaired glucose tolerance.
4. Reduced response to metyrapone test.
5. Reduced serum folate concentration.

MUTAGENESIS AND CARCINOGENESIS: Long-term, continuous administration of natural and synthetic estrogens in certain animal species increases the frequency of carcinomas of the breast, cervix, vagina, and liver.

PREGNANCY CATEGORY X: Estrogens should not be used during pregnancy. See **CONTRAINDICATIONS** and Boxed Warning.

NURSING MOTHERS: As a general principle, the administration of any drug to nursing mothers should be done only when clearly necessary since many drugs are excreted in human milk.

ADVERSE REACTIONS: The following have been reported with estrogenic therapy: changes in vaginal bleeding pattern and abnormal withdrawal bleeding or flow, breakthrough bleeding, spotting, increase in size of uterine fibromyomata, vaginal candidiasis, change in amount of cervical secretion; tenderness or enlargement of breasts; nausea, vomiting, abdominal cramps, bloating, cholestatic jaundice; chloasma or melasma that may persist when drug is discontinued, erythema multiforme, erythema nodosum, hemorrhagic eruption, loss of scalp hair, hirsutism; steepening of corneal curvature, intolerance to contact lenses; headache, migraine, dizziness, mental depression, chorea; increase or decrease in weight; reduced carbohydrate tolerance; aggravation of porphyria; edema; changes in libido.

ACUTE OVERDOSAGE: May cause nausea and vomiting.

DOSAGE AND ADMINISTRATION:

PREMARIN® Brand of conjugated estrogens tablets, USP

1. *Given cyclically for short-term use only.* For treatment of moderate-to-severe vasomotor symptoms, atrophic vaginitis, or atrophic urethritis associated with the menopause (0.3 mg to 1.25 mg or more daily). The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible. Administration should be cyclic (eg, three weeks on and one week off). Attempts to discontinue or taper medication should be made at three- to six-month intervals.
2. *Given cyclically:* Hypoestrogenism. Osteoporosis.
Hypoestrogenism due to: Female hypogonadism—2.5 mg to 7.5 mg daily in divided doses for 20 days followed by 10 day rest period. If bleeding does not occur by the end of this period, the same dosage schedule is repeated. Female castration or primary ovarian failure—1.25 mg daily, cyclically. Adjust upward or downward according to response of the patient. For maintenance, adjust dosage to lowest level that will provide effective control.
Osteoporosis—0.625 mg daily. Administration should be cyclic (eg, three weeks on and one week off).

PREMARIN® Brand of conjugated estrogens Vaginal Cream

Given cyclically for short-term use only. For treatment of atrophic vaginitis or kraurosis vulvae.

The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible.

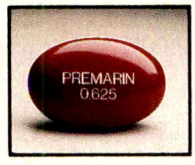
Attempts to discontinue or taper medication should be made at three- to six-month intervals.

Usual dosage range: 2 g to 4 g daily, intravaginally, depending on the severity of the condition.

Patients with an intact uterus who are treated with either PREMARIN Tablets or Vaginal Cream should be monitored for signs of endometrial cancer and appropriate measures taken to rule out malignancy in the event of persistent or recurring abnormal vaginal bleeding.

Revised August 21, 1989

PREMARIN®
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The appearance of this tablet is a trademark of Wyeth-Ayerst Laboratories.

OSTEOPOROSIS
The only cure is prevention

Reference: 1. Weiss NS, Ure CL, Ballard JH, et al: Decreased risk of fractures of the hip and lower forearm with postmenopausal use of estrogen. *N Engl J Med* 1980;303:1195-1198.

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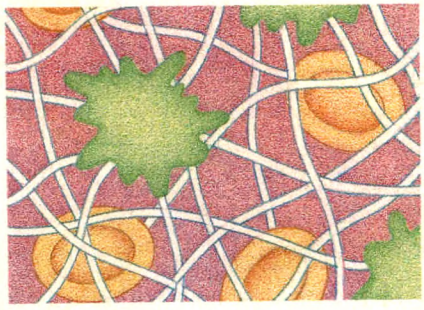
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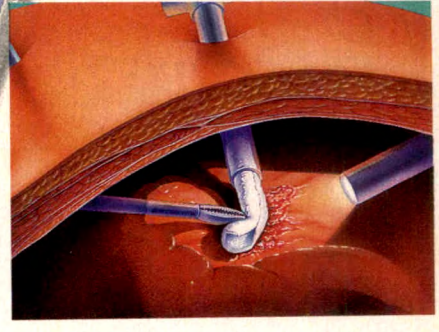
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Foundation Congress of the EUROPEAN SOCIETY FOR GYNECOLOGI AND OBSTETRIC INVESTIGATION (E.S.G.O.I.)

Madonna di Campiglio (Italian Dolomites, Trentino, Italy February 7 - 14, 1993

Founders: Pedro N. Barri Raguè (Barcelona, Spain), **Jean Cohen** (Paris, France), **Tom K.A.B. Esl** (Nijmegen, The Netherlands), **Andrea R. Genazzani** (Modena, Italy), **Lars Hamberger** (Götebc Sweden), **Joseph G. Schenker** (Jerusalem, Israel), **Hermann P.G. Schneider** (Münster, Germa **Markku Seppälä** (Helsinki, Finland), **John Studd** (London, United Kingdom).

The goal of the E.S.G.O.I. is to put more emphasis to investigations in both basic and clinical aspects of stetrics and Gynecology as well as arranging - every two years - a common "forum" for debates on perimental, pharmacological, technological, molecular, surgical and other ongoing researches in our cipline.

The ESGOI's Foundation Congress will be held in **Madonna di Campiglio,** in the magnificent scenery the Italian Dolomites, and organized with Plenary Sessions, Minisymposia, Slide and Poster Sessions. Scientific Committee's efforts will be directed to involve in the final program the most updated resea groups as well as a large number of contributions originating from the various European Departments Obstetrics and Gynecology.

A Preliminary Programme containing the list of Special Lectures, Plenary Sessions and Forums will mailed upon request. The list of the proposed topics for minisymposia, slide and poster sessions is follows:

- gynecological oncology
- infective diseases
- gynecological endocrinology
- reproductive medicine
- fetal and perinatal medicine
- obstetric care
- menopause
- senology
- immunology
- molecular biology
- assisted procreation
- endoscopic surgery
- new surgical techniques
- oncogenes and carcinogenesis
- urogynecology
- psychobiology in Obst/Gynec
- contraception
- sexuology
- anesthesia and analgesia
- chemotherapy
- sexually transmitted diseases
- ultrasound and color doppler

Deadline for abstract submission will be **November 30, 1992.** The text of the abstract should be typed bond paper, single spacing within a frame (size: 16 cm/6.4 inches width - 12 cm/4.8 inches height) indic ing: title, authors, affiliation and text.

For further scientific information and abstract submission please contact:

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Manuscript submission. Manuscripts should be submitted to one of the two Editors in Chief as follows:

1. **Dr. Zuspan**—manuscripts from the eastern and southeastern United States and from England, Israel, and Italy, manuscripts written for Clinical Opinion and Current Development, Letters to the Editors, and all manuscripts presented before one of the official sponsoring societies, except the Society for Gynecologic Investigation. *Manuscripts from The Society of Perinatal Obstetricians should be submitted to Dr. J. Peter Van Dorsten, P.O. Box 34, MCV Station, Richmond, VA 23298.*
2. **Dr. Quilligan**—manuscripts from states west of Pennsylvania, West Virginia, Kentucky, Tennessee, and Mississippi; manuscripts from Hawaii, Alaska, Canada, and abroad (except England, Israel, and Italy). Dr. Quilligan is responsible for manuscripts from the Society for Gynecologic Investigation. *Manuscripts from the Society for Gynecologic Investigation should be submitted to Dr. Roger A. Lobo, Women's Hospital, 1240 North Mission Road, Room 1M2, Los Angeles, CA 90033.*

Author's suggested reviewers. When authors submit their manuscripts, they must provide the names and addresses of at least three reviewers for consideration by the Editors.

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Checklist. The checklist that appears with the copyright statement in each issue of the JOURNAL must be fully completed by the author(s) and sent with each submitted manuscript. A more complete description of each item that must be checked is provided under the appropriate heading in the Information for Authors, which is published in each issue of the JOURNAL. *If the checklist is not completed in full, the deficiencies will be noted, and the form, as well as the manuscript when necessary, will be returned to the author for completion.*

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requisite authority. Human experimentation that requires local institutional approval must have this approval *before the experiment is started* and approval must be so indicated in the *Material and Methods* section of the submitted manuscript. Reports of experiments on nonhuman animals or other species must *state in the* Material and Methods *section of the manuscript* that the guidelines for the care and use of the animals *approved by the local institution* were followed. The type of nonhuman animals or other species used in an investigation must be named in the title, abstract, key words, and *Material and Methods* section of the manuscript.

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The original and two good-quality photocopies of the manuscript and three sets of black-and-white glossy prints of illustrations are required. See "Color figures" and "Computer-generated figures" for special requirements. Manuscripts must be submitted in English.

Manuscripts must be typed double-spaced on one side only of 22 × 28 cm (8½ × 11 inch) white bond paper with 1-inch margins at top, bottom, and sides. Number pages consecutively in the upper right-hand corner in the following order: title page, condensation, abstract, body of text, acknowledgments, references, figure legends, and tables.

Title page. The title page (page 1) should contain in sequence the title (concise and suitable for indexing purposes); author line with first name, middle initial, and last name of each author and each author's highest academic degree (both MD and PhD are acceptable, but honors degrees are not permitted); city(ies), state(s) in which the study was conducted; divisional, or departmental, and institutional affiliations at the time the study was performed; source(s) of financial support; presented line, if applicable; disclaimers, if any; name, address, business and home telephone numbers, and fax number of author to whom requests for reprints should be addressed (if reprints will not be available, it should be so stated); and name, address, business and home telephone numbers, and fax number of author responsible for correspondence concerning the manuscript if different from author to whom reprint requests are addressed. *At the bottom of the title page supply a short title for the running head not exceeding 52 characters (including word spaces).*

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Abstract page including key words/phrases. On manuscript page 3, type the abstract, double-spaced, with the required margins and headed by the title of the article and name(s) of author(s). Below the abstract, list 3 to 5 *key words or short phrases* for indexing purposes.

A structured abstract is required for certain types of articles and a *standard abstract format* is required for other types of articles; therefore the author(s) should select and prepare the appropriate abstract to be used in the submitted manuscript. This applies to both independently submitted and society manuscripts.

Structured abstract. A structured abstract, limited to 150 words, is to be used for regular articles and society articles; excluded are Clinical Opinion, Current Development, case report, and brief communication articles. The structured abstract is to contain the following major headings: *Objective(s); Study Design; Results;* and *Conclusion(s).* The *Objective(s)* reflects the purpose of the study, that is, the hypothesis that is being tested. The *Study Design* should include the setting for the study, the subjects (number and type), the treatment or intervention, and the type of statistical analysis. The *Results* include the outcome of the study and statistical significance if appropriate. The *Conclusion(s)* states the significance of the results.

Standard abstract. The standard abstract format is required for Clinical Opinion, Current Development, case report, and brief communication articles. The standard abstracts for Clinical Opinion and Current Development articles are to be 50 to 150 words and standard abstracts for case reports and brief communications are limited to 50 words. Even though the formal names of these four types of articles are not used, an author should determine his/her type of manuscript and prepare the appropriate standard abstract, regardless of whether it is to be submitted independently or as a part of a society's transactions.

Text. Do not hesitate to write your manuscript in the first person and active voice if they are more appropriate to the information you wish to convey. The passive voice is generally more effective for describing techniques or observations, since the emphasis is on the "action" rather than on the person performing the action.

Only standard abbreviations are to be used. Consult the

Estimating length of manuscripts

With the exception of Current Development articles, the length of text material (introduction through *Comment* section) in regular manuscripts accepted for publication normally ranges from 750 to 4200 words (an average of 2000 words). A text of 4200 words or more can seldom be accepted, especially if tables and figures are included. The average manuscript of 2000 words of text with abstract, 3 tables with captions, 2 figures with legends, and references makes a 5.7-page article in the JOURNAL. The 2000 words of text alone makes approximately 8 pages of manuscript typed double-spaced with the required 1-inch margins (approximately 250 words per page). A table or figure that occupies both columns of half a JOURNAL page is equivalent to approximately 500 words in manuscript. Thus, if a greater number of illustrations and tables is used, the length of the text should be adjusted accordingly.

Council of Biology Editors Style Manual or the AMA's *Manual of Style.* Abbreviations in the title are not acceptable. They should be avoided, if possible, in the abstract. In the text they should be kept to a practical minimum. The full term for which an abbreviation stands should precede its first use in the text unless it is a standard unit of measurement.

Either the generic, chemical, or proprietary names of drugs may be used. If the generic or chemical name is used, authors may, if they desire, insert the proprietary name in parentheses after the first mention in the text, with the name of the manufacturer and city and state.

Regular articles are customarily organized into the following sections: an introduction and headings that identify *Material and Methods*, *Results*, and *Comment*. Authors may wish to summarize their findings in a short paragraph at the end of the *Comment* section. This format may not be appropriate for some types of articles.

In the introduction, state concisely the purpose and rationale for the study and cite only the most pertinent references as background.

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In the *Results* section present the detailed findings. Include mentions of all tables and/or figures. Figures and tables should supplement, not duplicate, the text; presentation of data in either one or the other will suffice. Emphasize only your important observations; do not compare your observations with those of others. Such comparisons and comments are reserved for the *Comment* section.

In the *Comment* section state the importance and significance of your findings but do not repeat the details given in the *Results* section. Limit your opinions to those strictly indicated by the facts in your report. Compare your findings with those of others. No new data should be presented in this section.

Acknowledgments. Acknowledge only persons who have made substantive contributions to the study.

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Examples (if six or fewer authors, list all; if seven or more authors, list three then et al.):

JOURNAL: Flamm BL, Dunnett C, Fischermann E, Quilligan EJ. Vaginal delivery following cesarean section: use of oxytocin augmentation and epidural anesthesia with internal tocodynamic and internal fetal monitoring. AM J OBSTET GYNECOL 1984;148:759-63.

BOOKS: Ledger WJ. Dystocia and prolonged labor. In: Wilson JR, Carrington ER, eds. Obstetrics and gynecology. St. Louis: CV Mosby, 1987 vol 8:474-93.

Personal communications and unpublished data, if essential, may be used but not as numbered references. If they are used, they are to be referred to, within parentheses, at the appropriate location in the text. If used, the author(s) must obtain written and signed permission for their use from the individual being quoted. *This signed permission must accompany the manuscript when it is submitted to the Editor.* Published abstracts can be used as numbered references; however, reference to the complete published article is preferred.

Figures. The term *figure* includes all types of illustrations such as graphs, diagrams, photographs, flow charts, and line drawings. A reasonable number of figures will be reproduced without charge, but special arrangements must be made with the Editors for color figures at an additional charge to the author. The quoted charge is per page of color, and as many as eight color figures can be included on one page if the color balance is appropriate.

Figures must be cited consecutively in the text in Arabic numerals and identified with a gummed label on the back of the figure with author(s) name(s), title of article, figure number, and top marked clearly. Consistency in size within the article is strongly preferred. Any special instructions regarding sizing should be clearly noted. Do not use paper clips or mar the surface of figures in any way. Figures will be returned only on request by the author.

For *black-and-white figures,* submit three sets of 3 × 4 inch (minimum) to 5 × 7 inch (maximum) unmounted, glossy prints. All lettering must be done with commercially available paste-on letters (or numbers) or by a professional; typed or freehand lettering is not acceptable. All lettering must be in proportion to the drawing, graph, or photograph. Original drawings, appropriately done in black India ink, roentgenograms, and other material must be submitted as glossy prints with good black-and-white contrast.

For *color figures* submit original transparencies and two sets of unmounted prints on glossy (smooth surface) paper. Polaroid prints are not acceptable. Color transparencies must have a color balance (consistency in lighting and film speed) that is acceptable to the author and Editors before acceptance for publication. Please note that 35 mm transparencies are enlarged to twice their original size. If it is important to deviate from this standard, please so indicate when the material is submitted. The top, first author's last name, and figure number must be indicated on the front of each transparency and the back of each print.

Computer-generated figures must be legible and clearly printed in jet black ink on heavy coated paper with either a glossy or dull finish. Any patterns or shadings must be dark enough for reproduction and must be distinguishable from each other. Lines, symbols, and letters should be both smooth and complete. The legend should not appear on the figure. On the back of each figure the name of the first author and the figure number should be given and the top indicated. Three original individual laser or plotter prints are to be submitted *unmounted* with the manuscript. Laser prints should be full size at 300 dots per inch (DPI) or greater full-page resolution; multiple illustrations on a page cannot be accepted. Dot matrix prints and photographic halftones are not acceptable. *Color figures* are acceptable, but special arrangements must be made with the Editors. The colors used must be dark enough and of sufficient contrast for reproduction. With the exception of fluorescent colors, all colors can be reproduced in four-color figures.The preparation and submitting of color prints should follow the preceding guidelines for black-and-white computer-generated illustrations.

Legends to figures. Legends for all figures must be typed together in numeric order double-spaced on one sheet of paper (or more if necessary) separate from the text of the manuscript. This page must be numbered in sequence after the references. Titles should be included in the legend, not on the print. Original magnifications should be provided. If a figure has been taken from copyrighted material, the legend must give full credit to the original source.

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Requirements for certain types of manuscripts

Case reports and brief clinical and basic science communications. Limit of 700 words, 2 references. Include *standard abstract format*, of 50 words maximum, 3 to 5 key words/phrases for indexing purposes, and short title. If tables and/or figures are used, an equivalent number of words must be deducted from the total (see "Estimating Length of Manuscript").

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CLINICAL SECTION

Clinical Articles

Continuous combined conjugated equine estrogen–progestogen therapy: Effects of medroxyprogesterone acetate and norethindrone acetate on bleeding patterns and endometrial histologic diagnosis

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London, England

OBJECTIVES: This study was designed to assess the incidence of amenorrhea with continuous combined therapy by using two different progestogens and to determine whether early bleeding predicts subsequent bleeding and endometrial response.

STUDY DESIGN: Seventy-nine postmenopausal women on sequential estrogen-progestogen treatment were switched to continuous combined estrogen-progestogen therapy comprising conjugated equine estrogens 0.625 mg daily with either norethindrone acetate 0.35 mg twice daily or medroxyprogesterone acetate 2.5 mg twice daily added continuously for 78 weeks. All bleeding was recorded, and endometrial biopsies were performed at 26 and 78 weeks.

RESULTS: Only one third of the women who starting the study had amenorrhea by week 78, but 46 (62%) of these women had withdrawn, mainly because of chronic irregular bleeding. Endometrial atrophy was observed in the majority of biopsy specimens. The two progestogens had similar effects. Bleeding patterns were useful predictors of subsequent bleeding, but not of endometrial response.

CONCLUSIONS: Persistent irregular bleeding is common with continuous combined estrogen-progestogen therapy. Women with persistent early bleeding should probably revert to sequential treatment. Regular endometrial sampling is advised. (AM J OBSTET GYNECOL 1992;167:1-7.)

Key words: Continuous combined estrogen-progestogen, endometrial bleeding

Progestogen addition to estrogen replacement therapy although desirable for endometrial protection,[1, 2] has certain disadvantages. Progestogens may cause physical and psychologic side effects,[3] partially reverse some of the beneficial lipid and lipoprotein effects seen with estrogen-only therapy,[4, 5] and with sequential addition cause regular uterine bleeding in approximately 85% of women without hysterectomies.[6, 7] Although the bleeding pattern can predict the endometrial response and thereby reduce the need for endometrial biopsy,[8] the recurrence of withdrawal bleeding is unacceptable to some women.

Continuous combined estrogen-progestogen therapy has been proposed to overcome or reduce these problems. Progestogens have antimitotic actions on the endometrium[9, 10]; if given continuously, progestogens should induce endometrial atrophy, which, in turn, will produce amenorrhea. Furthermore, with continuous administration lower daily doses should be effective; thus the unwanted physical, psychologic, and metabolic effects of progestogens may be reduced.

The published experience of continuous combined estrogen-progestogen therapy is, however, limited.[11] Long-term safety data are sparse, and only one report[12] has provided endometrial data on patients taking continuous combined estrogen-progestogen therapy for

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more than 12 months; 21 patients were studied at 18 months.

We have compared the effects of two commonly prescribed progestogens, norethindrone acetate and medroxyprogesterone acetate, when given as continuous combined therapy, on bleeding patterns and endometrial histologic diagnosis over an 18-month period. To clarify the effect of duration of therapy on the development of amenorrhea we separated our bleeding data into two time periods; weeks 1 through 25 and 26 through 78.

Material and methods

Seventy-nine postmenopausal women with intact uteri attending the Menopause Clinics at King's College Hospital and the Chelsea Hospital for Women were recruited and gave informed consent. All were taking oral estrogen replacement therapy continuously (every day), with a progestogen added for the first 10 to 12 days of each calendar month. All but one were taking conjugated equine estrogens 0.625 mg daily; the exception took piperazine estrone sulfate, and she was changed to conjugated equine estrogens at the beginning of continuous combined treatment. Forty-five patients were taking norethindrone acetate as their sequential progestogen, 19 medroxyprogesterone acetate, 11 norgestrel, 3 dydrogesterone, and 1 oral progesterone.

At enrollment all patients were either postmenopausal (at least 12 months since their last menstrual period) or had been receiving sequential therapy for relief of menopausal symptoms for ≥3 months. The majority had undergone endometrial biopsies during the previous 2 years. Two biopsy specimens showed mild cystic hyperplasia, and one showed secretory activity with an area of cystic change. All others showed normal histologic changes consistent with phase of treatment.

After enrollment patients continued with sequential estrogen-progestogen therapy for two more cycles for collection of baseline physical and psychologic data. All patients had regular withdrawal bleeding during these cycles. Six days after stopping the sequential progestogen patients started continuous combined estrogen-progestogen therapy and were allocated to either continuous norethindrone acetate 0.35 mg twice daily (morning and evening) or medroxyprogesterone acetate 2.5 mg twice daily in addition to the daily conjugated equine estrogens 0.625 mg. Continuous combined estrogen-progestogen therapy was administered for 78 weeks and patients were reviewed after approximately 12, 25, 52, and 78 weeks of continuous combined estrogen-progestogen therapy.

The intensity of all bleeding was recorded every day on a bleeding chart relative to the normal menstrual loss with a four-point scale; spotting, mild, moderate, or heavy. Patients also recorded when tablets were taken and noted any event that might provoke bleeding, such as concurrent antibiotic use.

Biopsy specimens were obtained either by dilation and curettage when the patient was under general anesthesia or by suction curettage (Vabra, Berkeley Medevices, Berkeley, Calif.) performed as an outpatient procedure. All tissue was placed immediately into formol–acetic acid for histologic examination. The features assessed were those of the normal menstrual cycle. Biopsies were performed after 25 and 78 weeks of continuous combined estrogen-progestogen therapy.

All treatment-related side effects were recorded. Various options to adjust treatment were incorporated into the protocol. If menopausal symptoms recurred after the patient changed to continuous combined estrogen-progestogen therapy, the clinician could increase the dose of conjugated equine estrogens. With chronic, irregular bleeding patients were given three options: (1) withdraw, (2) continue with current treatment, or (3) increase the dose of either the estrogen or the progestogen. The success of the latter was assessed by reviewing the bleeding records for the subsequent 6 weeks. If the bleeding was reduced or if amenorrhea developed, the change was regarded as successful; if the bleeding did not abate, the change was regarded as unsuccessful.

Statistical methods. Comparison of the demographic data between the two groups was made with unpaired Student's t test.

Results

Data from five patients were excluded. Two failed to complete the bleeding charts satisfactorily, two missed tablets on so many occasions that the bleeding pattern could not be interpreted, and one took the incorrect dose of medication. Thus analyzable data were available from 74 patients, 46 on norethindrone acetate and 28 on medroxyprogesterone acetate. There were no significant differences between the norethindrone acetate and medroxyprogesterone acetate groups for mean age (55.1 and 54.9 years, respectively), mean time since menopause (73.8 and 71.2 months), and mean weight (60.9 and 63.4 kg, respectively).

Reasons for discontinuation. Seventeen (23%) of the 74 patients withdrew during the first 25 weeks, and 29 (39%) withdrew between weeks 26 and 78. Thus 28 (38%) patients completed the study. The reasons are shown in Table I. The most common reason with both progestogens over the entire study was chronic bleeding. It is noteworthy that some of the patients who withdrew because of chronic bleeding were not necessarily those who experienced the longest bleeding.

Table I. Patients who withdrew from study according to type of progestogen and time period

Reason for withdrawal from study	Weeks 1-25				Weeks 26-78			
	Norethindrone acetate		Medroxyprogesterone acetate		Norethindrone acetate		Medroxyprogesterone acetate	
	No.	%	No.	%	No.	%	No.	%
Chronic bleeding	7	15*	4	14*	5	11	3	11*
Side effects of progestogen	3	7	0	0	4	9	2	7
Miscellaneous	0	0	2	7	8	17	4	14
Lost to follow-up	1	2	0	0	2	4	1	4
TOTAL	11	24	6	21	19	41	10	36

*Includes five patients (three receiving norethindrone acetate and two receiving medroxyprogesterone acetate) who also reported physical and psychologic progestogen-induced side effects.

Breast tenderness, fluid retention, and mood changes were the most common side effects of progestogen.

Over the entire study 14 patients withdrew for miscellaneous reasons; these included mildly elevated blood pressure, fibroids requiring hysterectomy, recurrence of menopausal symptoms not controlled by increasing the dose of conjugated equine estrogens, and breast cancer. The remainder of the miscellaneous withdrawals were not caused by side effects. Six patients violated the protocol, and four patients wanted to stop therapy for personal reasons such as anxieties about cancer. An additional four patients were lost to follow-up.

Treatment adjustments. Twenty-three patients underwent a total of 26 treatment adjustments. Five patients increased the dose of conjugated equine estrogens for symptom recurrence: this was successful in four. Twenty-one adjustments were made for bleeding: 13 were increases in the dose of conjugated equine estrogens, 4 increases in the dose of norethindrone acetate (3 to 0.7 mg twice daily, 1 to 1.5 mg twice daily) and 4 increases in the dose of medroxyprogesterone acetate to 5 mg twice daily. Seven, three, and one of these adjustments, respectively, were successful.

Eight of the patients who completed the study underwent at least one treatment adjustment; 20 of the patients who completed the study took the same medication throughout the study.

Percentage of patients with amenorrhea. To assist with the expression and comprehension of these data, the study has been divided into 6-week intervals. Any bleeding considered attributable to missed tablets or concomitant antibiotic administration or that occurred within 14 days of an endometrial biopsy was excluded.

The percentage of patients in the two progestogen groups reporting amenorrhea at each 6-week interval throughout the study is shown in Fig. 1 and is expressed as a percentage of patients remaining in the study and as a percentage of patients who started the study.

Bleeding episodes. For the two phases of the study (weeks 1 through 25 and 26 through 78) the total days of bleeding was calculated for each patient. Each day on which any bleeding was reported was defined as one bleeding episode.

A total of 1944 days of bleeding was recorded during weeks 1 through 25 and 1091 days during weeks 26 through 78. Bleeding was scored as moderate or heavy on 130 (6.6%) and 59 (5.4%) days, respectively. The largest amount of bleeding experienced by any patient was 161 days in weeks 1 through 25 and 127 days in weeks 26 through 78. The patterns in the two progestogen groups were similar. (Further data are available on request.)

Table II reports the relationship between the total duration of bleeding experienced during the first 25 weeks and that reported between weeks 26 through 78. The 17 patients who withdrew during the first 25 weeks are not included; one patient who withdrew at the beginning of week 26 has also been excluded.

Endometrial histologic diagnosis. Biopsies were performed on 51 of the 57 patients who completed the first 25 weeks and on 17 of the 28 patients who completed week 78. The results for the two progestogens are shown in Table III. No hyperplasia or carcinoma was observed in any sample (including those in which the tissue was too scant for reliable interpretation). In spite of thorough curettage, a sample inadequate for reliable interpretation or "no tissue" was obtained on 54 occasions.

Six patients completed week 25 but did not have a biopsy performed; three patients declined biopsies and withdrew from the study because of chronic bleeding. Two refused biopsy but wished to continue in the study; one of these subsequently underwent a biopsy procedure at week 78. Outpatient curettage was not possible in one patient at week 25 because of cervical stenosis; she declined dilation and curettage.

Mild cystic hyperplasia had been diagnosed in three patients at biopsy before continuous combined estrogen-progestogen therapy; at week 25 late secretory en-

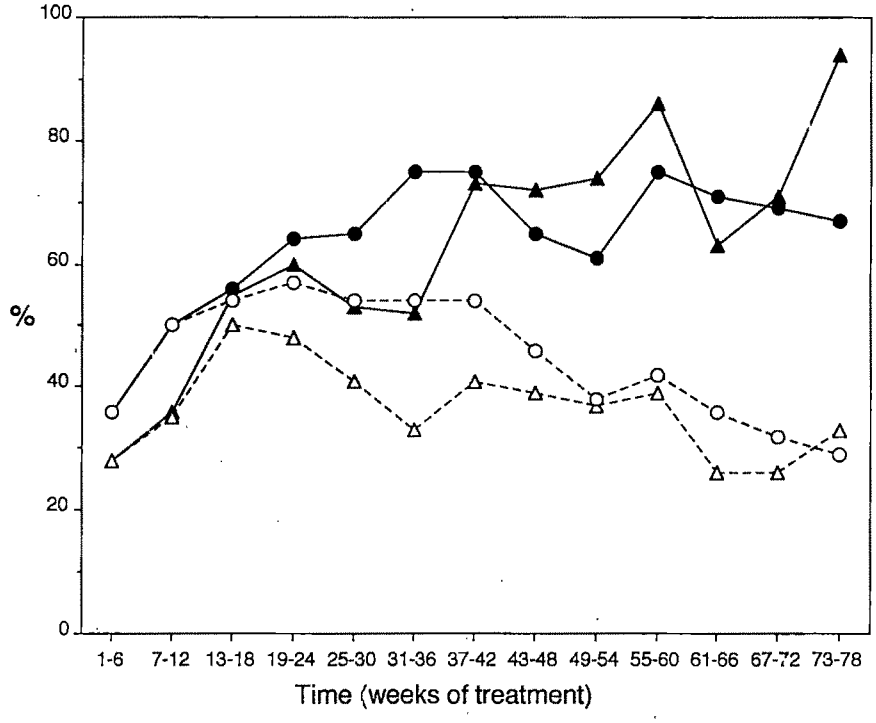


Fig. 1. Percentage of women on a regimen of norethindrone acetate and medroxyprogesterone acetate with amenorrhea by 6-week treatment intervals shown as a percentage of patients remaining in the study (▲——▲, norethindrone acetate; o——o, medroxyprogesterone acetate) at each time point and as a percentage of patients who started the study (Δ - - - - Δ, norethindrone acetate; o - - - - o, medroxyprogesterone acetate).

Table II. Duration of bleeding reported during weeks 1 through 25 and subsequent bleeding experienced in weeks 26 to 78

Weeks 1-25		*Weeks 26-78*			
		Duration of bleeding (days)			
Duration of bleeding (days)	*No. of patients*	*0*	*1-9*	*10-59*	*≥60*
Amenorrhea	10	10	0	0	0
1-9	17	13	2	2	0
10-59	21	3	8	7	3
≥60	8	0	2	2	4

Data for norethindrone acetate and medroxyprogesterone acetate groups are combined. Only includes patients who completed 26 weeks (17 withdrew in weeks 1 through 25; 1 withdrew in week 26).

dometrium was diagnosed in one of these patients and atrophic endometrium was diagnosed in another. The third patient withdrew at 12 weeks because of progestogen-induced side effects.

Eleven patients declined the second biopsy. Between weeks 26 and 78, 8 had had amenorrhea, 1 had spotted for 7 days, and 2 had experienced prolonged bleeding (68 and 118 days).

Relationships between endometrial histologic diagnosis and duration of bleeding. Because norethindrone acetate and medroxyprogesterone acetate produced similar histologic results for the two phases of treatment (Table III), the data for the two progestogens have been combined. In Table IV the histologic results are presented relative to the number of days of bleeding experienced during the first 25 weeks and between weeks 26 and 78. The second number is an identical expression of the histologic results but related to the total number of days of bleeding recorded during the 6-week interval immediately before the biopsy.

Comment

Only 28 patients completed the study; these numbers were too small for statistical comparisons between the norethindrone acetate and medroxyprogesterone acetate groups. Although four patients were lost to follow-up and 10 of the 14 miscellaneous withdrawals were not caused by side effects (Table I), 32 (43%) patients discontinued the study because of problems with treatment. This contrasts with our recent studies of sequential regimens, also of 18 months' duration,[13] in which only 15% of women receiving oral and 3% receiving transdermal sequential therapies discontinued treatment because of side effects.

The most common problem was chronic irregular bleeding (Table I). Although the majority of the bleed-

Table III. Histologic features according to type of progestogen and time of biopsy

	Treatment regimen (phase)							
	Norethindrone acetate (wk 25)		*Medroxyprogesterone acetate (wk 25)*		*Norethindrone acetate (wk 78)*		*Medroxyprogesterone acetate (wk 78)*	
Histologic diagnosis	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
Proliferative	2	6.5	0	0	0	0	0	0
Secretory	5	16	1	5	1	11	0	0
Atrophic	2	6.5	2	10	0	0	1	12.5
Inadequate sample—no tissue	22	71	17	85	8	89	7	87.5
TOTAL	31		20		9		8	

Table IV. Relationship between endometrial histologic diagnosis at weeks 25 and 78 and bleeding pattern between weeks 1 through 25 and 26 through 78, respectively.

	No. of samples showing histologic diagnosis							
	Inadequate sample—no tissue		*Atrophic*		*Secretory*		*Proliferative*	
Duration of bleeding	*Total before biopsy*	*Total during 6 wk before biopsy*	*Total before biopsy*	*Total during 6 wk before biopsy*	*Total before biopsy*	*Total during 6 wk before biopsy*	*Total before biopsy*	*Total during 6 wk before biopsy*
Weeks 1-25								
Amenorrhea	8	30	1	3	1	3	0	0
1-9	13	7	1	0	2	2	1	1
10-59	16	2	2	1	1	1	0	1
≥60	2	0	0	0	2	0	1	0
Weeks 26-78								
Amenorrhea	5	10	0	1	1	1	0	0
1-9	4	4	1	0	0	0	0	0
10-59	4	1	0	0	0	0	0	0
≥60	2	0	0	0	0	0	0	0

ing was spotting or light, it could be very prolonged. Four patients with bleeding for >60 days in either time period completed the study, but we doubt whether such perseverance will occur in a routine clinical environment.

Various strategies for reducing the incidence of bleeding have been proposed. Because norethindrone acetate and medroxyprogesterone acetate are rapidly cleared from plasma, we administered these progestogens twice daily to try to provide more constant plasma levels. Our results show that this is not more successful than once daily administration. Staland,[14] Mattson et al.[15] and Hargrove et al.[16] all reported increased incidence of bleeding among perimenopausal women. Only five of our women were within 12 months of their last menstrual period; the mean duration since menopause was approximately 6 years. However, like 93% of those in the study of Magos et al.,[12] all our patients had received sequential therapy before commencing continuous combined estrogen-progestogen therapy. Magos et al.[12] reported dropout rates of between 20% and 49%, depending on the estrogen dose; these rates are comparable with those reported here but are higher than those 0% to 33% rates reported with 9 to 12 months of continuous combined estrogen-progestogen therapy in women previously untreated for at least 4 weeks.[14-21]

Various explanations for the higher rates of discontinuation have been observed by Magos et al.[12] and us. Sequential therapy may have a carryover effect that increases the frequency and duration of irregular bleeding after the change to continuous combined estrogen-progestogen therapy. However, if this carryover effect is the mechanism, it is difficult to understand biologically why it should be so prolonged. Between weeks 26 and 78, chronic irregular bleeding occurred not infrequently and resulted in eight withdrawals (Table I). Alternatively, patients well established on sequential therapy who experience regular withdrawal bleeding may be less prepared than previously untreated women to have chronic, irregular bleeding. Additionally, continuous as compared with sequential administration of the progestogen can cause adverse symptomatic and psychologic changes (Table I), and four patients suffered recurrences of their original symptoms with continuous combined estrogen-proges-

togen therapy, probably because of progestogenic antagonism of estrogen benefits.

The last alternative is that various authors have expressed their results differently. Although there is agreement that the percentage of patients with amenorrhea increases over time (Fig. 1) we believe that because of the high proportion of dropouts resulting from chronic bleeding, data on the incidence of amenorrhea should be displayed not only relative to the numbers of patients remaining in the study but also relative to the numbers starting the study. In this study persistent amenorrhea was eventually achieved by only approximately one third of patients (Fig. 1) who started continuous combined estrogen-progestogen therapy.

None of our attempts to reduce bleeding were uniformly successful, and these results add to the already-confusing literature on the optimal strategy for patients with chronic bleeding. Some authors have reported better results with higher progestogen doses,[12, 17] but others have reported the converse.[20]

There was no evidence of endometrial disease in this study. The majority of biopsies at both time periods resulted in "no tissue," a sample too small for reliable assessment or endometrial atrophy (Table III). Endometrial histologic diagnosis could not be predicted reliably from the bleeding pattern, although complete amenorrhea was only associated with endometrial atrophy or with secretory endometrium (Table IV).

We regard proliferative endometrium with continuous combined estrogen-progestogen therapy as indicating a lack of progestational effect. Over a long term this might increase the risk of endometrial carcinoma. This individual variation in response to the added progestogen, with some patients exhibiting a good effect (i.e., atrophy) but others none, is well recognized with sequential therapies.[22] Proliferative endometrium was diagnosed twice at the week 25 biopsy. One patient had experienced only 1 day of spotting up to week 25 and thereafter had amenorrhea. The second patient discontinued treatment at week 61, having bled 282 days.

Our data suggest that whereas amenorrhea indicates progestational activity within the endometrium, durations of bleeding of 1 day can be associated with lack of endometrial response to the administered progestogen. We leave the reader to draw the conclusions about the need for biopsy in patients with minimal bleeding.

Women who have amenorrhea from the outset are likely to continue to have it, and the majority of those with bleeding for <10 days during the first 25 weeks of therapy will also have amenorrhea as the duration of treatment is extended. In those whose bleeding is of moderate duration (10 to 59 days) during the first 25 weeks, it is more difficult to predict future bleeding, which can be variable (Table II). Conversely, in those with chronic bleeding during the first 25 weeks it is likely to continue and amenorrhea is unlikely. These women may be best advised to switch to sequential therapy.

We thank Mrs. Angela Short for her help in preparing this manuscript.

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Macrosomia in postdates pregnancies: The accuracy of routine ultrasonographic screening

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OBJECTIVE: The purpose of this study was to assess the efficacy of routine ultrasonographic assessment of fetal weight in predicting fetal macrosomia in postdates pregnancies.
STUDY DESIGN: A total of 519 pregnancies of ≥41 weeks' duration were subjected to ultrasonographic estimation of fetal weight within 1 week of delivery. Estimated fetal weights were compared with birth weights. Linear regression analysis was performed and prediction limits for estimated fetal weights were generated.
RESULTS: Twenty-three percent of infants had birth weights ≥4000 gm and 4% had birth weights ≥4500 gm. The mean percent absolute error was 7.7%. At a birth weight of >3750 gm, the Hadlock model (which uses abdominal circumference and femur length) systematically overestimated the birth weight. The sensitivity, specificity, and positive and negative predictive values for the ultrasonographic diagnosis of macrosomia were 56%, 91%, 64%, and 87%, respectively. The prediction limits generated determine the range of birth weights predicted for a given estimated fetal weight.
CONCLUSION: Routine ultrasonographic screening for macrosomia in postdates pregnancies is associated with a relatively low positive predictive value. (AM J OBSTET GYNECOL 1992;167:7-11.)

Key words: Postdates pregnancies, fetal macrosomia, ultrasonographic prediction of fetal weight

Fetal macrosomia is a condition that may be associated with significant obstetric morbidity. The macrosomic fetus is at elevated risk for birth asphyxia and birth trauma; the mother carrying a fetus with macrosomia may be at increased risk of both operative delivery and postpartum hemorrhage.[1-4] Factors that predict fetal macrosomia include maternal weight gain of >20 kg, maternal obesity, diabetes, and antecedent pregnancies that have been delivered ≥7 days post term.[1, 2]

Postterm pregnancies have traditionally been considered to be at elevated risk of adverse outcome and have therefore been subjected to fetal surveillance. Approximately one fifth of all pregnancies continue beyond 41 weeks' gestation,[5] and postdate pregnancies represent a significant proportion of patients undergoing fetal surveillance.[6] These surveillance programs have focused on the detection of fetal hypoxia associated with uteroplacental insufficiency.[7] To this end fetal heart rate monitoring, biophysical profile, and assessment of amniotic fluid volume have been used. The presence of fetal macrosomia, however, with its attendant risk to

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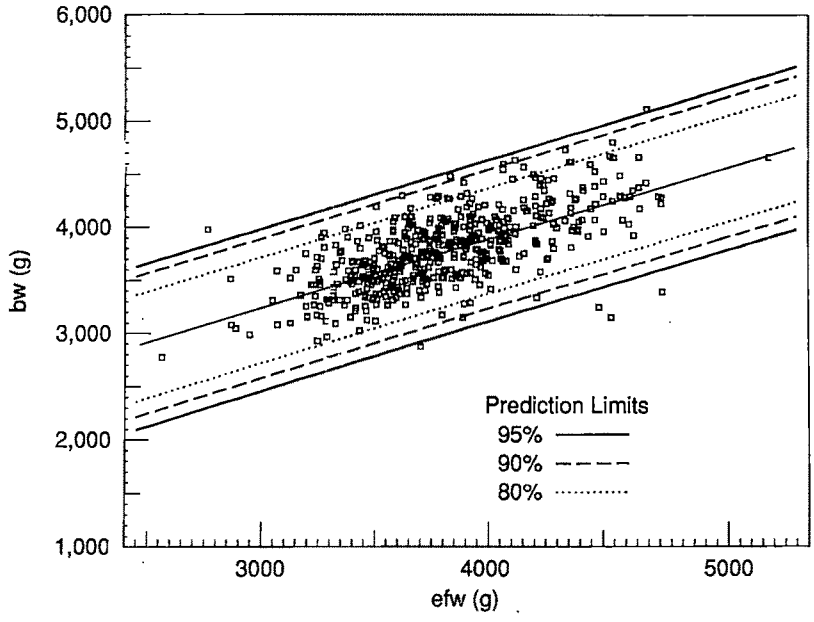


Fig. 1. Correlation of birth weight and estimated fetal weight.

mother and fetus, has not been sought on a routine basis.

Chervenak et al.[8] have recently proposed that ultrasonographic estimation of fetal weight may be useful in identifying macrosomic fetuses and should be incorporated into the surveillance of the postterm fetus. The identification of the macrosomic fetus may provide the clinician with important information that may alter the delivery management. The purpose of our study was to prospectively evaluate the accuracy of ultrasonographic fetal weight estimation in a population at elevated risk for fetal macrosomia, namely patients with postterm pregnancies. We also proposed to develop prediction limits to assist in the clinical interpretation of ultrasonographic fetal weight estimations.

Material and methods

Between January 1989 and September 1990 all patients seen for fetal surveillance because of prolonged pregnancy at the Weiler Hospital of the Albert Einstein College of Medicine underwent ultrasonographic estimation of fetal weight, assessment of amniotic fluid volume, and nonstress testing. A total of 519 patients were delivered within 1 week of fetal weight estimation. These patients formed the study group. Only patients with singleton, uncomplicated pregnancies of >41 weeks' gestation were included in the study. Gestational age was based on the last menstrual period and early ultrasonographic examination when available. Gestational age was based on ultrasonographic examination only when there was a discrepancy of >10 days between menstrual dates and ultrasonographic dates during the first trimester of pregnancy. After the initial visit all patients were evaluated semiweekly.

Ultrasonographic examination was performed with a real-time scanner (Aloka 650, Corometrics, Wallingford, Conn.) with a 3.5 mHz linear array probe. Measurements of biparietal diameter, abdominal circumference, and femur length were performed with standard techniques.[9] The estimated fetal weight based on abdominal circumference and femur length was derived from the tables of Hadlock et al.[10] Actual birth weights were then compared with estimated fetal weight. Percentage differences between estimated fetal weight and actual birth weight were calculated for each patient. The sensitivity, specificity, and positive and negative predictive values of an estimated fetal weight of ≥4000 gm to predict a birth weight of ≥4000 gm were determined. Regression analysis of birth weight on estimated fetal weight and calculation of 70th, 80th, 90th, and 95th percentile prediction limits were calculated with statistical software (GBSTAT 2.0, Dynamic Microsystems, Silver Spring, Md.) (see Appendix).

Results

Five hundred nineteen patients referred for antenatal surveillance because of postdates pregnancy were delivered within 1 week of ultrasonographic estimation of fetal weight. The mean estimated fetal weight was 3640 ± 461 gm (mean ± SD). One hundred three (19.9%) fetuses were estimated to weigh ≥4000 gm; 24 (4.6%) were estimated to weigh ≥4500 gm. The mean

Table I. Prediction limits for ultrasonographic estimates of fetal weight

Estimated fetal weight (gm)	*Predicted birth weight (gm)*	*Percentile*			
		70th	*80th*	*90th*	*95th*
3500	3578	3174-3982	3081-4075	2939-4217	2817-4340
3750	3741	3337-4145	3244-4238	3102-4380	2980-4502
4000	3903	3499-4308	3406-4402	3265-4543	3142-4665
4250	4066	3661-4471	3568-4564	3426-4706	3303-4829
4500	4229	3823-4634	3730-4728	3588-4870	3465-4993
4750	4391	3985-4798	3891-4891	3748-5034	3626-5157
5000	4554	4147-4961	4053-5055	3910-5198	3786-5322

birth weight of these infants was 3670 ± 446 gm (mean ± SD). One hundred nineteen (22.9%) infants weighed ≥4000 gm and 21 (4.1%) weighed ≥4500 gm.

The mean absolute error of the ultrasonographic estimated fetal weight, defined as the absolute value of birth weight − estimated fetal weight, was 279 gm, and the mean percent absolute error, defined as |birth weight − estimated fetal weight/birth weight| × 100, was 7.6% ± 6.8% (mean ± SD). Of weight estimates, 41.6% were within 5% of the actual birth weight, 72.5% were within 10%, and 89.4% were within 15%. The sensitivity, specificity, and positive and negative predictive values of an estimated fetal weight ≥4000 gm in predicting macrosomia (i.e., birth weight ≥4000 gm) were 56%, 91%, 64%, and 87%, respectively. When the estimated fetal weight used to predict macrosomia was increased to ≥4500 gm, the sensitivity, specificity, and positive and negative predictive values were 15%, 99%, 81%, and 80%, respectively.

These data describe the accuracy of ultrasonographic estimation of fetal weight when applied to our study population. To assess the accuracy of the ultrasonographic prediction of macrosomia when applied to an individual patient, prediction limits were generated. A regression of birth weight and estimated fetal weight with the associated 80th, 90th, and 95th percentile prediction limits is shown in Fig. 1. This regression is described by the following equation: $Y = 1302.062 + (0.6504)X$, where Y = birth weight and X = estimated fetal weight. The correlation coefficient of this equation is 0.6731, and the relationship is significant ($p = 0.0001$).

With this regression line the birth weight associated with a given estimated fetal weight can be predicted. The predicted birth weight and the 70th, 80th, 90th, and 95th percentile prediction limits associated with a given estimated fetal weight are shown in Table I. In infants whose birth weight exceeded approximately 3750 gm the Hadlock model, which uses abdominal circumference and femur length to estimate fetal weight, systematically overestimated the actual birth weight. The prediction limits show that for a given estimated fetal weight a range of predicted birth weights may be derived. The width of this range is dependent upon the level of confidence placed in the prediction. Thus the range of the 95th percentile prediction limit is substantially greater than that of the 70th percentile prediction limit. Table I indicates that the predicted birth weight associated with an estimated fetal weight of 4500 gm was 4229 gm. This estimated fetal weight was associated with a predicted birth weight ranging from 3823 to 4634 gm 70% of the time. Similarly, 95% of the time the same estimated fetal weight was associated with a predicted birth weight ranging from 3465 to 4993 gm.

Comment

Although the increased morbidity and mortality associated with postdate pregnancy has long been appreciated, only recently has the contribution of macrosomia to these adverse outcomes been understood.[8] Boyd et al.[1] noted that the incidence of fetal macrosomia increases with increasing gestational age. These results have been confirmed by Arias,[2] who suggested that ultrasonographic assessment of fetal weight be incorporated into the surveillance of postdates pregnancies. Chervenak et al.[8] have shown that the presence of fetal macrosomia increases with increasing gestational age and that this may be associated with a significant increase in cesarean sections performed for labor disorders. Those authors have focused attention on the fact that postponing delivery in postdates pregnancies may ultimately increase the incidence of fetal macrosomia, thereby increasing the rate of operative delivery.

The implementation of a screening program for fetal macrosomia is predicated on two assumptions. First, it must be assumed that the techniques used in screening can accurately predict fetal macrosomia. Second, implementation of a screening program presupposes that the accurate prediction of macrosomia mandates a well-defined plan of management. Our study examines the validity of the first assumption. Although clinical esti-

mation of fetal weight and measurement of symphyseal-fundal height have long been used to diagnose fetal macrosomia, the accuracy of these techniques is limited.[10-19] Ultrasonographic estimation of fetal weight with the formula of Hadlock et al.[10] has been shown to be the most effective formula when used in late pregnancy.[17] This formula is also particularly well suited for use in the postdates pregnancy because it makes use of abdominal circumference and femur length. We have previously shown that because of the position of the fetal head, the biparietal diameter may not be measurable in as many as one fifth of postdates patients subjected to ultrasonographic examination.[8] Although the formula of Hadlock et al.[10] may be superior to other formulas, our data show it too can be associated with a significant overestimation. The mean percent absolute error observed with this formula was 7.7%. We have observed that only 56% of fetuses with macrosomia were identified as such on the basis of the ultrasonographic weight estimation. When a fetus was diagnosed as having macrosomia on the basis of an ultrasonogram, the birth weight was found to be >4000 gm in only 64% of cases.

The inherent inaccuracies of the ultrasonographic techniques used to diagnose fetal macrosomia have placed the clinician in a most unenviable position. In the presence of an ultrasonographic diagnosis of macrosomia the clinician may opt for a trial of labor with its attendant risk of labor abnormalities and birth trauma. Alternately, an abdominal delivery with its attendant morbidity can be chosen as long as it is appreciated a priori that in 36% of cases the antenatal diagnosis of macrosomia will not be substantiated.

An informed clinical interpretation of an estimated fetal weight must integrate a variety of parameters, including the sensitivity, specificity, and positive and negative predictive value of the test in predicting fetal macrosomia. This interpretation must also reflect that as the estimated fetal weight increases, so does the likelihood that a birth weight of >4000 gm will be observed. The range of birth weights predicted for a given estimated fetal weight is an additional parameter of clinical relevance. This range is referred to as the prediction limit. For a given estimated fetal weight a 90th percentile prediction limit provides the range of birth weights observed 90% of the time. The prediction limits shown in Table I represent a clinically useful method of relating ultrasonographic estimated fetal weights to expected birth weights. This table indicates that to be 70% confident that a predicted birth weight will be >4000 gm, an estimated fetal weight of approximately 4750 gm is required. Of the five hundred nineteen patients studied, only 1% had an estimated fetal weight of >4750 gm.

In conclusion, we have demonstrated that prospective ultrasonographic screening in a patient population at elevated risk of macrosomia is associated with a rather low positive predictive value. Therefore implementation of such screening is of limited use. Further improvements in the techniques used to predict fetal macrosomia must occur before the introduction of such screening programs.

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Appendix

Calculation of prediction limits. The $(1 - \alpha)$ percent prediction interval for an individual predicted birth weight based on an estimated fetal weight is defined by:

$$\hat{Y}_{n+1} \pm t_{n-2,\alpha/2} \sqrt{\left[1 + \frac{1}{n} + \frac{(X_{n+1} - \overline{X})^2}{\sum_{i=1}^{n} (X_i - \overline{X})^2}\right] S_e^2}$$

where $\hat{Y}_{n+1}$ is the predicted birth weight and X_{n+1} is the corresponding estimated fetal weight.

S_e, an estimate of the SD of the birth weights about the regression line, is equal to:

$$\sqrt{\frac{\Sigma(Y_i - \hat{Y}_i)^2}{n - 2}}$$

The X_i and $\overline{X}$ represent the estimated fetal weights and average estimated fetal weights, respectively, for our sample size n. $t_{n-2,\alpha/2}$ is the Student t distribution value with $(n - 2)$ degrees of freedom.[20]

The clinical diagnosis of asphyxia responsible for brain damage in the human fetus

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OBJECTIVE: Our objective was to review the clinical findings in infants who died in the perinatal period with brain damage attributable to asphyxia.
STUDY DESIGN: The neuropathologic findings in 208 perinatal deaths have been reviewed. Thirty cases (22 fetal, eight newborn) had evidence of white matter or neuronal necrosis due to asphyxia. The clinical course of the pregnancy in 22 cases with brain damage attributable to fetal asphyxia were examined.
RESULTS: The diagnosis of asphyxia was confounded by several factors: (1) asphyxia may occur at any time in the last half of pregnancy, (2) 50% of the antepartum asphyxia occurred when the pregnancy had no risk factors, (3) periodic fetal assessment in the complicated preterm pregnancies failed to identify the asphyxial episodes in the remaining cases of antepartum asphyxia, and (4) indicators of fetal asphyxia in the cases of intrapartum fetal asphyxia were obtained after the central nervous system injury had occurred.
CONCLUSION: These findings highlight the difficulty in the diagnosis of fetal asphyxia at a stage that could permit intervention to prevent brain damage. (Am J Obstet Gynecol 1992;167:11-5.)

Key words: Fetal asphyxia, brain damage, clinical diagnosis

Studies in the monkey and lamb have demonstrated that fetal asphyxia can cause central nervous system injury and corresponding motor and cognitive deficits in surviving animals.[1-4] However, there is no consensus as to how much of the burden of neurologic handicap in the community can be attributed to perinatal asphyxia in humans.[5] Much of this continuing dilemma is due to the absence of a specific diagnostic marker for fetal asphyxia; this absence hampers a reliable interpretation of the data derived from epidemiologic studies.

The introduction of risk scoring and the assessment of fetal behavior has improved the identification of the fetus at risk for asphyxia, although blood gas and acid-base assessment of fetal blood can provide a specific diagnosis in some cases. However, in spite of these developments, there is no evidence that these clinical and technologic improvements have led to a decrease in the incidence of cerebral palsy.[6, 7]

An accurate description of the neuropathology that is responsible for motor and cognitive deficits in children is an important step in our progress to the understanding and prevention of cerebral palsy and men-

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Table I. Criteria for defining interval between asphyxial insult and neuropathologic condition observed at death

Time	*Pathologic criteria*
18 to 48 hr	*Neuronal necrosis* Immature neurons: Nuclear pyknosis and karyorrhexis Mature neurons: Nuclear pyknosis and cytoplasmic eosinophilia
<72 hr	Appearance of white matter macrophages or microglia
>72 hr	Appearance of reactive astrocytes in white matter
>4 hr	Macroscopic cavitation

tal retardation. Although the neuropathologic abnormalities associated with these disasters in children and young adults have been well described, it has been virtually impossible to confidently attribute the structural changes in the brain to events that may have occurred during the perinatal period, many years before the death of the patient. What has been missing are studies that attempt to correlate clinical abnormalities that are observed in the fetus and newborn infant with the neuropathologic abnormalities in those who die. Such a study would give some idea of the incidence of these severe insults and would allow markers of asphyxia to be identified.

To examine these questions we have reviewed the clinical findings in a series of infants who died in the perinatal period and in whom brain damage attributable to asphyxia had occurred. Preliminary data from this study have been used to establish the timing of sublethal asphyxial insults accounting for the brain damage in the fetus and newborn.[8] The information presented in the current report represents an expansion of these studies and is particularly concerned with an analysis of the clinical data that might allow the clinician to identify those asphyxial insults that are likely to lead to perinatal brain damage.

Methods

This was a retrospective clinicopathologic study of 208 perinatal deaths between 1976 and 1989 in whom a satisfactory neuropathologic examination was performed. The identification of asphyxia was based on the neuropathologic features characteristic of the effect of this insult on the central nervous system. There was evidence of neuronal or white matter necrosis in 30 cases (14%).

A chart review was carried out. The maternal, obstetric, fetal, labor, delivery, and newborn complications in each case were recorded. Particular attention was paid to the timing of the onset of labor and delivery. Fetal assessments performed in the antepartum period, during labor, and at delivery were reviewed in detail.

Criteria have been established that indicate the approximate time of the asphyxial insult based on the neuropathologic changes in the central nervous system at the time of death.[9] Infants who die during or immediately after an asphyxial insult have structurally normal brains because changes have not had time to occur; survival for ≥18 hours is required before histologic changes become apparent. Table I summarizes these changes, which follow a sequence starting with neuronal necrosis at 18 hours and progressing through microglial and astrocytic activation to the formation of white matter cavities at approximately 96 hours after the asphyxial insult.

The neuropathologist (D.A.R.) had no knowledge of the clinical data during the assessment of the neuropathology. An estimated interval between the initial asphyxial insult and the neuropathologic condition present at the time of death was determined on the basis of the criteria listed in Table I. Consequently, the time at which the asphyxial insult occurred could be placed in one of the antepartum, intrapartum, or newborn periods. Subsequently, the clinical events that occurred before and at the predicted time of brain damage were then examined.

Results

The initial asphyxial insult that led to brain damage was estimated to have occurred in the antepartum period in 17 cases, in the intrapartum period in five cases, and in the newborn period in eight cases. This report examines the 22 cases of fetal asphyxia. Although the neuropathologic criteria do not indicate the time of the asphyxial insult precisely, it was estimated that the antepartum asphyxial insult occurred >48 hours before the onset of labor in three term and eight preterm pregnancies and between 12 and 24 hours before the onset of labor in six preterm pregnancies. Intrapartum fetal asphyxia was estimated to have occurred in two term and three preterm pregnancies.

Table II summarizes the data concerning the timing of the asphyxial insult and the duration of the clinical complications for eight cases from the antepartum asphyxia group in whom the initial asphyxial insult appears to have occurred when the pregnancy was clinically normal. The asphyxial episode occurred >3 days before the onset of labor in three term pregnancies with no clinical risk factors. In three preterm pregnancies the asphyxial episode preceded an antepartum hemorrhage due to premature placental separation by several hours to several days. The asphyxial episode preceded preterm labor in two pregnancies by 12 hours and 7 days.

Table III summarizes the data for nine preterm pregnancies in whom the antepartum asphyxial insult appeared to have occurred during a clinically complicated pregnancy. The pregnancy complications in each case included one or more maternal, obstetric, or fetal com-

Table II. Antepartum asphyxia group in whom initial asphyxial insult preceded clinical indicators of pregnancy complications

	Perinatal death	*Time of asphyxia before death*	*Clinical problem*	
			Duration	*Complication*
Baby B (41 wk, 3600 gm)	Stillbirth	72 hr	—	None
Baby C (40 wk, 3100 gm)	Stillbirth	>4 days	—	None
Baby V (40 wk, 2680 gm)	Stillbirth	>4 days	—	None
Baby R (34 wk, 2250 gm)	Stillbirth	72 hr	9 hr	Antepartum hemorrhage, premature placental separation
Baby S (30 wk, 1160 gm)	Neonatal death at 4 hr	<48 hr	3 hr	Anterpartum hemorrhage, premature placental separation
Baby H (29 wk, 1640 gm)	Neonatal death at 2 hr	>72 hr	15 hr	Antepartum hemorrhage, premature placental separation
Baby G (26 wk, 1020 gm)	Neonatal death at 47 hr	>4 days	10 hr	Preterm labor
Baby T (25 wk, 640 gm)	Neonatal death at 7 hr	<48 hr	12 hr	Preterm labor

Table III. Group with antepartum asphyxia in whom asphyxial insult occurred in a complicated pregnancy

	Perinatal death	*Time of asphyxia before death*	*Clinical problem*		*Fetal assessment*
			Duration	*Complication*	
Baby F (33 wk, 2690 gm)	Stillbirth	< 48 hr	16 days	Maternal pancreatitis	FHR
Baby H (twin B) (32 wk, 1070 gm)	Stillbirth	<72 hr	21 days	PIH, IUGR	FHR, BPP
Baby B (33 wk, 980 gm)	Neonatal death at 10 hr	<72 hr	28 days	PIH, IUGR	NST, BPP
Baby R (twin A) (29 wk, 3600 gm)	Neonatal death at 1 hr	>72 hr	28 days	Hydramnios, nonimmune hydrops	NST, BPP
Baby R (twin B) (29 wk, 940 gm)	Neonatal death at 72 hr	>72 hr	28 days	IUGR	NST, BPP
Baby B (twin A) (28 wk, 620 gm)	Neonatal death at 50 hr	>72 hr	14 days	IUGR	FHR
Baby B (twin B) (28 wk, 580 gm)	Neonatal death at 7 hr	<72 hr	14 days	IUGR	FHR
Baby A (27 wk, 1260 gm)	Neonatal death at 72 hr	>72 hr	24 hr	Antepartum hemorrhage	FHR
Baby P (25 wk, 560 gm)	Neonatal death at 1 hr	<48 hr	6 wk	Antepartum hemorrhage	FHR

FHR, Fetal heart rate auscultation; *BPP,* biophysical profile; *PIH,* pregnancy-induced hypertension; *IUGR,* intrauterine growth retardation.

plications. A conservative management program was in effect in each case to achieve improved fetal maturity for periods up to 6 weeks before either spontaneous or elective preterm delivery. Fetal surveillance during this period included intermittent fetal heart rate auscultation in five cases and serial biophysical profile and nonstress tests (NST) in four cases. No distinctive abnormality of fetal behaviour was observed at the estimated time of the sublethal asphyxial insult. An abnormal biophysical profile reported in two cases was recorded after the estimated time of the asphyxial insult and central nervous system injury. This sequence is exemplified by the following case.

A 28-year-old obstetric patient, gravida 1, para 0, was admitted to the hospital at 28 weeks' gestation because of moderate preeclampsia. An ultrasonographic diagnosis of growth retardation was made at 30 weeks. At 32 weeks an ultrasonographic examination reaffirmed the diagnosis of growth retardation with decreased amniotic fluid volume. Neuropathologic studies at postmortem examination indicated that the asphyxial insult occurred at this time, i.e., 72 hours before delivery. However, serial NSTs continued to be reactive. A nonreactive NST and an abnormal biophysical profile were first observed on the day of delivery, leading to emergency cesarean section. The 980 gm newborn had a significant metabolic acidosis, pH 7.02 and buffer base 29 mmol/L, and died 10 hours after delivery.

Table IV summarizes the data for five pregnancies in which the asphyxial insult was estimated to have occurred during the intrapartum period. The two term

Table IV. Asphyxia group in which estimated time of asphyxial insult was during intrapartum period

			Intrapartum assessment		
				Umbilical arterial blood gas	
	Duration of labor (hr)	*Perinatal death*	*Fetal heart rate*	*pH*	*Buffer base (mmol/L)*
Baby F (41 wk, 3350 gm)	3	Neonatal death at 72 hr	Bradycardia		NA
Baby J (41 wk, 3350 gm)	12	Neonatal death at 72 hr	Bradycardia		NA
Baby J (36 wk, 2540 gm)	7	Neonatal death at 14 hr	↓ Variability Deceleration	6.95	27
Baby V (30 wk, 1500 gm)	6	Neonatal death at 21 hr	Deceleration	6.64	<10
Baby L (27 wk, 700 gm)	5	Neonatal death at 11 days	Deceleration	7.07	29

NA, Not available.

pregnancies had been uneventful. However, during labor one patient had meconium in the amniotic fluid, and the second patient had a prolapsed cord with a breech presentation. Complications in the preterm pregnancies included antepartum hemorrhage in two cases and pregnancy-induced hypertension in one case. All cases had evidence of clinical fetal distress as expressed by fetal bradycardia, decreased baseline heart rate variability, or decelerations during the latter part of labor. Fetal asphyxia was confirmed in three cases with blood gas and acid-base measurement at delivery. The Apgar scores in these cases were 1 and 1 minute and 1 to 4 at 5 minutes.

Comment

The fetus in each of these 22 pregnancies had experienced, sometime before birth, a "sublethal" asphyxial insult of sufficient severity to cause brain damage but not of such severity to lead to the immediate death of the fetus. The prevalence of "sublethal" asphyxial episodes is not known, but the incidence of neuropathologic abnormalities in this series of perinatal deaths (14%) provides an estimate of the frequency of such asphyxial episodes. Wigglesworth[10] in a recent study of neuropathologic findings in mature stillbirths has reported an incidence of damage to gray matter in 33% of normally formed third trimester stillbirths. It is apparent from current imaging of parenchymal lesions in the newborn, that similar episodes may occur in the fetus, and that these abnormalities may account for motor and cognitive deficits in the surviving children.[11, 12]

Moderate to severe periventricular leukomalacia was observed in 14 of the 22 cases of fetal asphyxia. All cases had some degree of neuronal necrosis in the cerebral cortex, basal ganglia, thalamus, or brain stem. These findings are in keeping with previous descriptions in the literature.[13-16]

Such asphyxial insults may occur at any time during the last half of pregnancy or during the course of labor. Antepartum insults, occurring from 12 hours to 7 days before the onset of labor, accounted for 75% of the cases of fetal asphyxia. The stress of labor with intrapartum fetal asphyxia of sufficient severity to cause neuropathologic abnormalities occurred in 25% of the subjects studied.

These findings indicate that the clinician must anticipate asphyxial insults at any time during the last half of pregnancy before or during labor. This study clearly demonstrates that identification of impending fetal asphyxia and thus timely intervention is hampered by the frequent covert onset of asphyxia and the limitations of current methods of fetal assessment. Thus eight cases of antepartum asphyxia occurred when there were no apparent indicators that the fetus was at risk. Of these, three episodes occurred in uncomplicated term pregnancies and five episodes occurred in preterm pregnancies before the appearance of clinical evidence in either antepartum hemorrhage or preterm labor.

The NST and the biophysical profile are widely acknowledged measures of fetal well-being. However, these noninvasive measurements are intermittent and, in the preterm pregnancy before 28 weeks, they are difficult to interpret. Approximately half of the antepartum asphyxial insults reported in this study occurred in complicated preterm pregnancies during conservative management programs to improve fetal maturity. The clinical observations at the histologically estimated time of the asphyxial insult did not suggest a compromised fetus. The abnormal NST and biophysical profile recorded in two cases were obtained

after the asphyxial insult and central nervous system injury had occurred.

Intrapartum fetal assessment cannot begin until the patient is admitted to the hospital, and certain criteria are met to justify such surveillance. Thus assessment does not begin with the onset of labor in most cases and may only be available for the latter part of labor in some cases. Intrapartum continuous fetal heart rate monitoring provides predictive criteria for fetal asphyxia, although the diagnosis can be confirmed by means of a blood gas and acid-base assessment of fetal blood during labor or at delivery. Such observations were obtained in the five cases in the intrapartum asphyxia group. However, it is likely the sublethal asphyxial insult accounting for the brain damage began before the onset of these observations of clinical fetal distress during labor and the blood gas measures at delivery.

These findings highlight some of the problems for the clinician in the diagnosis of fetal asphyxia at a stage that would permit intervention to prevent the neuropathologic conditions responsible for deficits in children.

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Simplified Gram stain interpretive method for diagnosis of bacterial vaginosis

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OBJECTIVE: Two methods which only consider bacterial morphotypes and require counting various types of bacteria, for interpreting Gram stains of vaginal secretions for the purpose of diagnosing bacterial vaginosis were previously described. A new interpretative method, which combines clue cells with bacterial morphotypes as diagnostic markers, is offered.

STUDY DESIGN: One hundred twenty patients were tested with the new interpretative method and clinical criteria of bacterial vaginosis as the reference standard.

RESULTS: The new method was found comparable to older methods when all were compared with clinical diagnostic methods. The sensitivity of the clue cell method was 86.1% and the specificity was 92.8%. The predictive value of a positive test was 83.7% for a population with a prevalence of bacterial vaginosis of 30%. The negative predictive value was 94.0%.

CONCLUSION: If clue cells are present and the nonlactobacilli morphotypes exceed lactobacilli morphotypes, bacterial vaginosis can be diagnosed without the need for exact bacterial counts. (Am J Obstet Gynecol 1992;167:16-9.)

Key words: Bacterial vaginosis, Gram stain, diagnosis

Bacterial vaginosis is the syndrome thought to be the most prevalent cause of vaginitis.[1] Several clinical diagnostic criteria, Gram stain methods, and biochemical markers have been developed to aid in diagnosis.[2] Gram stain of vaginal secretions is a relatively rapid, objective, and inexpensive method for diagnosing bacterial vaginosis by identifying the characteristic change in the vaginal flora.[3] It offers the advantage of allowing retrospective diagnosis. The normal vaginal flora consists of a wide variety of bacteria with lactobacilli as a major constituent. In bacterial vaginosis there is a greater than 400-fold increase in anaerobic flora with a diminished proportion of lactobacilli.[2] On Gram stain large gram-positive lactobacilli morphotypes are easily distinguishable from gram-negative organisms, cocci, *Gardnerella*, and other anaerobic bacterial morphotypes.[3] However, the change in proportion of lactobacilli appears to be an early stage in the development of bacterial vaginosis, as evinced by the low specificity of this single observation in wet mount preparations when compared with clinical diagnostic criteria for bacterial vaginosis.[4] In contrast, clue cells, vaginal epithelial cells that are so covered by attached bacteria that the cell borders become obscured, have been shown to be extremely specific for bacterial vaginosis defined by clinical criteria.[2, 4]

Existing Gram stain interpretive methods focus only on quantification of bacterial morphotypes.[3, 5] However, clue cells can also be identified on Gram stains.[6] The purpose of this study was to examine a Gram stain interpretive method involving mere observation of clue cells coupled to identification of predominant morphotype. This new interpretive method was compared with both preexisting methods, Spiegel et al.[3] and Nugent et al.[5] The clue cell method obviates the need for exact counts of various morphotypes and, because of the size of lactobacilli and clue cells, can be read at a magnification of ×400.

Methods

Gram stains were made from the vaginal secretions of 120 consecutive patients previously tested for and found not to have chlamydia (chlamydiazyme, Abbott Laboratories, Abbott Park, Ill.) or gonorrhea (culture, Thayer-Martin Agar, Becton Dickinson, Cockeysville, Md.) attending the Adolescent Pregnancy Program at Sinai Samaritan Medical Center. The mean age of these 120 patients was 16.1 ± 2.4 years, and the mean weeks of gestation was 19.1 ± 3.8. All teens were pregnant and enrolled at their initial obstetric appointment after informed consent was obtained. One slide was prepared for each patient. Commercial Gram stain reagents (Difco, Detroit) were used as instructed by the

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manufacturer. Each slide was interpreted by the same individual (P.J.O.) with three different Gram stain criteria.[3, 5] The criteria of Spiegel et al.[3] are described in Table I. Slides were read on a Microstar IV binocular microscope (Reichert, Cambridge Instruments Inc., Buffalo, N.Y.). A technician was selected to interpret the Gram stains, because this would most likely be the situation in a clinical laboratory setting and this is the group that the test is designed for. The criterion of Nugent et al.[5] is described in Table II. For the clue cell method, slides were read at a magnification of ×400 with a thin film of oil and a coverslip used to protect the objective lens. No exact enumeration was made. Bacteria in each field were evaluated simply as to whether lactobacilli morphotypes exceeded other morphotypes, and presence of clue cells was noted. Patients were considered to have bacterial vaginosis by the clue cell method of interpretation if nonlactobacilli morphotypes exceeded lactobacilli in the majority of fields examined and if clue cells were seen in at least two of 20 fields. Vaginal epithelial cells covered with lactobacilli were not considered clue cells. Cell edges had to be completely covered with bacteria to be called clue cells. Both conditions, presence of clue cells and predominance of nonlactobacilli, had to be observed for the Gram stain to be interpreted as bacterial vaginosis.

Patients were also evaluated for bacterial vaginosis by their examining physician (J.L.T., G.E.T., or J.A.J.) with the clinical diagnostic criteria proposed by Amsel et al.[7] Patients were considered clinically to have bacterial vaginosis if three of the following four characteristics were observed: clue cells in wet mount preparations of vaginal secretions, fishlike amine odor upon alkalinization of vaginal secretions with 10% potassium hydroxide, vaginal pH >4.5, and presence of homogeneous discharge that adhered to the walls of the vaginal introitus. The individual who was interpreting Gram stains (P.J.O.) was blinded from the clinical diagnosis. Corrected χ^2 statistics were used to compare the different interpretive methods.

Table I. Spiegel et al.[3] Gram stain interpretation

Morphotypes per field *	*Evaluation*
<1	1+
1-5	2+
6-30	3+
>30	4+

The following morphotypes were counted individually: large gram-positive rods were considered *Lactobacillus* morphotypes, short gram-variable rods were *Gardnerella* morphotypes, and cocci, curved rods, and fusiforms were other morphotypes. The patient was diagnosed as having bacterial vaginosis if *Lactobacillus* morphotypes were <2+, *Gardnerella* morphotypes were 4+, and at least one other morphotype was present.

*Average number of morphotypes per oil immersion field (original magnification, ×1000). Twenty fields were counted.

Table II. Nugent et al.[5] Gram stain interpretation

Score	*Lactobacillus*	*Gardnerella*	*Mobiluncus*
0	4+	0	0
1	3+	1+	1-2+
2	2+	2+	3-4+
3	1+	3+	
4	0	4+	

For the Nugent et al. method, exact morphotype counts were made as for the Spiegel et al. method at a magnification ×1000 but an additional morphotype, crescent rods (*Mobiluncus* morphotypes), was used in place of "other morphotypes." A score was given to each evaluation. The patient was diagnosed as having bacterial vaginosis if the score for all three morphotypes was >6.

Results

Thirty-six patients were found to have bacterial vaginosis by Amsel's clinical criteria. These patients were used as our reference standard as "true" positives. Comparison of Gram stain interpretative criteria to this reference standard is shown in Table III. Thirty-nine patients had bacterial vaginosis by Spiegel et al.'s Gram stain interpretive criteria, only 30 of which were true positive. The Nugent et al. Gram stain interpretive criteria identified 34 patients as having bacterial vaginosis, but only 31 were true positive. The clue cell Gram stain interpretive criteria identified 37 patients as having bacterial vaginosis, but only 31 were true positive. All 30 true-positive results by Spiegel et al. were also positive by Nugent et al. and clue cell interpretive criteria. In addition, both Nugent et al. and clue cell methods identified one additional true positive. The Nugent method had the least number of false-positive results.

Five patients having bacterial vaginosis by Amsel clinical criteria were falsely negative by all three Gram stain interpretive methods. The slides all showed approximately equal numbers of lactobacilli and other morphotypes, with two of the five (40%) showing clue cells. They were all considered intermediate, i.e., point value 4 to 7, by the criteria of Nugent et al. Neither Spiegel et al.'s nor clue cell methods of interpretation have an intermediate classification.

The sensitivities, specificities, and positive and negative predictive values with the clinical criteria of Amsel et al. as the reference standard are shown in Table III. There were no statistically significant differences between any of the three interpretative methods.

Comment

Comparing the three Gram stain interpretation methods to Amsel et al.'s clinical criteria shows that they all give comparable results. Simply screening the Gram stains for predominance of lactobacilli and absence of clue cells or for predominance of nonlactobacilli mor-

Table III. Evaluation of three Gram stain interpretive methods to diagnose bacterial vaginosis* ($N = 120$)

Method	True positive	True negative	False negative	False positive	Sensitivity		Specificity		Positive predictive value		Negative predictive value	
					Sample estimate (%)	SE (%)	Sample estimate (%)	SE (%)	Sample estimate (%)	SE (%)	Sample estimate (%)	SE (%)
Spiegel et al.	30	75	6	9	83.3	6.2	89.2	3.4	76.9	6.7	92.5	2.9
Nugent et al.	31	81	5	3	86.1	5.8	96.4	2.0	91.2	4.9	94.2	2.5
Clue cell	31	78	5	6	86.1	5.8	92.8	2.8	83.7	6.1	94.0	2.6

*Thirty-six women clinically diagnosed with bacterial vaginosis.

photypes and presence of clue cells is much easier than the more exact interpretive criteria outlined by Spiegel et al. and more recently by Nugent et al.[3, 5] The sensitivities of all methods are virtually identical. The Nugent method is probably more specific. Failure to demonstrate significance between the difference in specificities may have been due to small sample size. However, it is questionable whether the small increased specificity justifies the additional requirement to read slides at a magnification of ×1000 and to enumerate morphotypes. The finding that the Spiegel interpretive method had a specificity of 83% and sensitivity of 89% when compared with Amsel criteria is ±4% of the values reported by Eschenbach et al.[8] The clue cell method described should be more easily standardized for practitioners and laboratory personnel. Clinically, this method accounts for the fact that it is simplier to differentiate lactobacilli morphotypes from other types of bacteria. In addition, this simplified method does not require the examiner of the slide to spend large amounts of time to singularly count each various bacterial type involved with this syndrome as is described by other methodologies.[9]

Photomicrographs of Gram stains are not included because excellent photomicrographs showing clue cells and the change in bacterial flora seen in bacterial vaginosis have been published by both Spiegel et al. and Nugent et al.[3, 5] Both groups failed to consider clue cells in their interpretive criteria. Dunkelberg[6] described a method to diagnose bacterial vaginosis (he called it "*Hemophilus vaginalis* vaginitis") on the basis of clue cells "with an overlay of large numbers of small gram negative bacilli and equally dense bacterial population adjacent to the epithelial cells in gram-stained smears." His recommendation was not followed. His description was vague and not easily interpretable. The present report clearly defines exactly what the observer is to look for.

The recommended number of clue cells required to make the diagnosis of bacterial vaginosis is controversial. Eschenbach et al.[8] recommends that greater than 20% of epithelial cells must be clue cells. They claim that with this criterion 19% of patients that meet the Amsel clinical criteria do not have clue cells. However, if any well defined clue cell is used as the criterion, only 1% of patients that meet the Amsel criteria cannot be shown to have clue cells.[4] But both of these reports were based on wet mount observations of clue cells, not Gram stains. The specific recommendation of two fields with any clue cell worked well in this study. Whether this is the optimal number remains to be determined.

Early researchers claimed that clue cells did not correlate with bacterial vaginosis, basing this claim on successful culture of the organism now known as *Gardnerella* as their reference standard.[10] It has become well established that culture for *Gardnerella* does not correlate with clinical diagnosis of bacterial vaginosis.[8] Patients that have *Gardnerella* in their vaginal flora do not necessarily have bacterial vaginosis even when the organism is present in high numbers.[2, 8]

In contrast, clue cells have been shown to be highly sensitive and specific for bacterial vaginosis when clinical diagnostic criteria are used as the reference standard.[4] Including clue cells in the interpretive criteria obviates the need for enumeration of morphotypes, making the interpretation easier. This should allow greater interlaboratory reproducibility. The clue cell Gram stain interpretive criteria contains no intermediate grouping as is the case with the Nugent method because the significance of this group of patients is unknown.

The clue cell method offers a major advantage over other Gram stain interpretative methods for bacterial vaginosis. With this method a magnification ×400 facilitates the scanning of more fields of a patient's slide. Twenty fields at ×400 allows the examiner to view the entire area under the coverslip, which in turn allows the examiner to view a larger sample of the specimen, leaving less chance for error in making a diagnosis. Finally, this methodology takes much less overall labor time when compared to the other methods discussed, whether a clinician or laboratory personnel is involved. With less time used per patient specimen, larger numbers of women could be effectively screened, thereby promoting overall better patient care.

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Ethically justified guidelines for family planning interventions to prevent pregnancy in female patients with chronic mental illness

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OBJECTIVE: This article proposes ethically justified clinical guidelines for family planning interventions to prevent pregnancy in female patients.
STUDY DESIGN: We reviewed literature on family planning and consequences of pregnancy in patients with chronic mental illness and related that literature to ethical principles.
RESULTS: Patients with chronic mental illness are ethically unique because they have chronically and variably impaired autonomy. Existing guidelines and proposals for family planning interventions for mentally retarded patients are shown not to apply to such patients.
CONCLUSION: Three sets of guidelines for three groups of patients, representing the continuum of chronically and variably impaired autonomy, are proposed: (1) a set of guidelines for patients who can achieve thresholds of autonomy, (2) a set of guidelines for patients irreversibly near thresholds of autonomy, and (3) a set of guidelines for patients irreversibly below thresholds of autonomy. These guidelines should contribute significantly to the quality of obstetric and gynecologic care for female patients with chronic mental illness. (AM J OBSTET GYNECOL 1992;167:19-25.)

Key words: Ethics, family planning, chronic mental illness, chronically and variably impaired autonomy

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Family planning interventions to prevent pregnancy in female patients with chronic mental illness pose unique ethical challenges to the obstetrician and gynecologist. Respect for the patient's autonomy, a central ethical principle, is especially difficult to implement in the clinical setting of chronic mental illness. Whereas the definition of chronic mental illness is still evolving, established criteria include the diagnosis of a major

mental disorder such as schizophrenia, major affective disorder, or personality disorder; disabilities in social, physical, or employment-related areas; and a prolonged duration (usually ≥2 years) of treatment and illness.[1] Patients with chronic mental illness differ from the mentally retarded in a manner that ethics is uniquely qualified to identify and assess. Hence, existing guidelines and previous work on the ethics of family planning for mentally retarded patients are not conceptually adequate for patients with chronic mental illness. Guidelines of The American College of Obstetricians and Gynecologists for mentally handicapped patients[2] appear to treat informed consent issues in terms of a dichotomy between those capable of consent and those not capable of consent. Patients with chronic mental illness often fall between the extremes of this dichotomy and thus must be treated as an ethically and clinically distinct group.

To this end, we introduce and develop the concept of chronically and variably impaired autonomy as uniquely characterizing most patients with chronic mental illness. On the basis of this concept we present ethically justified, clinically applicable guidelines for family planning interventions for patients with chronic mental illness. These clinical guidelines include respect for the autonomy of these patients and attempts to restore autonomy when it is chronically and variably impaired. Women with chronic mental illness are at risk for not receiving adequate family planning and other obstetric and gynecologic services. Adopting the proposed guidelines may help to improve the quality of obstetric and gynecologic care of these women.

Clinical considerations

Chronic mental illness. Up to 2.4 million people are seen in community mental health clinics[3] whereas schizophrenia itself has a lifetime prevalence of about 1%.[4] Many of these patients are homeless and recidivistic, and their mental disability may limit their ability to seek local, state, or federal assistance such as housing, food stamps, rehabilitation, or protective services.[5] These patients also often have unrecognized physical illness,[6,7] and the course of illness may be complicated by substance abuse, including alcohol abuse, more frequently than chance would predict.[8,9] They may present to family planning clinics or gynecologic offices when brought by family members or referred by psychiatrists for contraception. Alternatively, patients may be referred when already hospitalized for an unrelated problem, or contraceptive issues may be identified when the patient presents for management of pregnancy.

Although a heterogeneous population, patients with schizophrenia may suffer from delusions, prominent hallucinations, or incoherence.[10] Alternatively, patients with bipolar mood disorder who are in a manic state demonstrate flight of ideas, grandiose and overgeneralized thinking, and easy distractibility.[11] Patients' ability to process information may also be limited by cognitive and attentional deficits.[12] Moreover, cognitive impairment, poor judgment, affective instability, and impulsivity are likely to result in behaviors that include unsafe sexual practices and intravenous drug use.[13] Thus patients with chronic mental illness are at risk for human immunodeficiency virus infection[13] and other sexually transmitted diseases, as well as for unwanted pregnancies.[14-16] They are also unlikely to comply with a recommendation of abstinence as a family planning strategy.

Maternal risks. Psychosis itself may directly affect maternal health when patients act on delusions or on auditory hallucinations.[17,18] Patients with chronic mental illness have also been found to be significantly less likely to have attended prenatal clinic appointments.[19] Psychotic denial of pregnancy, although a rare phenomenon, may relate to a failure to obtain prenatal care, as well as to postpartum emotional disturbance in the mother and precipitate or unassisted delivery.[20] For all pregnant women there is a small added risk of first admission for psychosis during pregnancy and within 3 months post partum.[21] A history of psychosis might therefore predispose to mental health complications for women during pregnancy.[22,23]

Fetal risks. Factors that may negatively influence pregnancy outcomes among this population include the possible effect of psychiatric medication and illicit drug use on the fetus.[24-26] Results have been mixed, however, regarding the relationship between mental illness and obstetric outcome. In two controlled studies no relationship was found.[27,28] On the other hand, one prospective study found an increased incidence of fetal and neonatal deaths among offspring of schizophrenics when compared with a closely matched control group.[29] In another controlled study, prematurity, lower birth weight, and lower apgar scores were found to relate to the severity and chronicity of mental illness.[30] Outcome can be improved, however, by close prenatal observation, including psychiatric hospitalization, which allows for appropriate obstetric intervention.[31]

Risks to possible future children. Possible future children may be at increased risk for a variety of chronic mental illnesses.[32-34] In schizophrenia, for example, the risk that a child of a schizophrenic parent will have schizophrenia has been shown to be increased. This increased risk is multifactorial and genetic, and environmental influences may account for it.[35-37] Children of the chronically mentally ill are frequently not raised by their mothers,[16] which would expose such children to the effects of early separation from an original parent. Little is known about whether childbearing pro-

tects the health of mentally ill women or predisposes them to mental illness.[38-40]

Decision-making about health care by the chronically mentally ill

The psychiatric impairments of these patients imply that they can and do experience difficulty in making decisions about their health care, including family planning interventions to prevent pregnancy. Decision-making about health care can be understood to involve the following process on the part of the patient, a process in which each step presumes successful completion of its predecessors:

1. The patient attends to the information disclosed to him or her by the physician.
2. The patient absorbs, retains, and recalls the information disclosed.
3. The patient appreciates that this information has significance in the patient's life and the lives of others (e.g., parents, spouse, partner, the fetus being taken to term, or future child). This requires that the patient understands that decisions about health care have consequences for the future and reasons well enough to connect present decisions with future possible consequences. This can be called *cognitive understanding*[40a] and requires some nominally intact cognitive abilities.
4. The patient evaluates those consequences on the basis of her values and beliefs. This can be called *evaluative understanding*[40a] and requires some nominally integrated sense of oneself.
5. The patient at some meaningful level expresses or communicates both cognitive and evaluative understanding.
6. The patient should be able at some meaningful level to express or communicate a decision based on such understanding.

This account differs from the usual account of the informed consent process, which is typically understood to involve disclosure of an adequate amount of information to the patient, understanding of that information by the patient (step 3), and a voluntary decision by the patient (step 6). Appelbaum and Grisso,[41] for example, identify four steps: "communicating choices," which blends without distinguishing steps 5 and 6; "understanding relevant information," which presupposes step 2 and is similar to step 3; "appreciating the situation and its consequences," which is also similar to step 3; and "manipulating information rationally," which is similar to step 4. Their approach does not clearly distinguish cognitive from evaluative understanding and ignores altogether step 1, a crucial consideration in the care of patients with chronic mental illness. We will comment on each of these six steps in some greater detail.

Steps 1 and 2 are included because patients with a history of chronic mental illness may have problems during the disclosure of information. These problems can result from factors such as attentional deficits that are associated with the patient's illness (e.g., auditory hallucinations or paranoia that may distract the patient from attending to, absorbing, recalling, or retaining what is disclosed).

Steps 3 and 4 are included to identify two related but distinct aspects of understanding. This distinction is important for informed consent involving patients with chronic mental illness because such illness can affect cognitive and evaluative understanding independently and in different ways. Cognitive understanding can be adversely affected by cognitive deficits. These tend to involve processing information (step 3) more than memory (step 2). For example, difficulties with step 3 may arise when patients use the mechanism of denial excessively in relation to their likelihood to engage in sexual intercourse.[14] Evaluative understanding can also be adversely affected. A patient's values and the ability to base decisions on them can be disrupted by paranoid or grandiose ideas, delusions, or affective disturbances such as significant depression. For example, patients may have a psychotic paranoia about oral contraceptives. Patients may also evaluate their ability to be adequate parents in an unrealistically positive way.[42-44]

Steps 5 and 6 are included because chronic mental illness frequently involves disrupted ability to express or communicate cognitive or evaluative understanding. This disruption can result from incoherence, circumstantiality, tangentiality, and loose associations.

Chronically and variably impaired autonomy

The obstetric, pediatric, and psychiatric outcomes described include the possibility of significant obstetric and mental health complications, possible adverse consequences in child development, and potential adverse consequences for patients with chronic mental illness and their partners. The decision-making process for these patients includes many factors that can impair or disrupt that process. It is a natural tendency for physicians, given these circumstances, to approach the family planning interventions for chronically mentally ill, fertile, sexually active female patients in a "paternalistic" fashion. By "paternalistic" we mean that the physician is inclined to strongly influence or even take over the patient's autonomous decision-making process to avoid significant adverse consequences.

Recall that autonomous decision-making may usefully be conceptualized in terms of the six-step decision-making process described. In discussing those six steps, we noted that for patients with chronic mental illness one or more of the capacities described is likely to be impaired and that this is a permanent feature of the

mental illness. In addition, this impairment may vary for each of the steps to differing degrees, including differing degrees over time in the same patient. We term this clinical ethical phenomenon *chronically and variably impaired autonomy.*

This phenomenon uniquely applies to the patient with chronic mental illness. In particular, it does not apply to severely and profoundly mentally retarded patients. This is because their loss of autonomy is nearly complete and does not vary. Nor does chronically and variably impaired autonomy apply to mildly and moderately mentally retarded patients, because they can participate in the consent process. This dichotomy is reflected in the guidelines of The American College of Obstetricians and Gynecologists.[2] The autonomy of patients with chronic mental illness typically falls along a continuum between those two extremes and thus must be treated separately.

Put another way, a chronically mentally ill patient may at any one time be above a threshold for one capacity (e.g., to attend to what is being said or cognitive understanding), while being below a threshold for another capacity (e.g., evaluative understanding or communicating a decision based on cognitive and evaluative understanding). That patient's decisions are indeed substantially autonomous,[45] but only in some respects and not in others, with variation over time.

We share the usual presumption, in ethics and law, that the patient possesses adequate decision-making capacity.[45] This capacity may seem, at first, to be called into question by the chronically and variably impaired autonomy. However, the variability of chronically and variably impaired autonomy calls into question both that the capacity to make decisions is always absent for such patients and that attempts at informed consent can be justifiably omitted. This helps to illuminate further the unique character of chronically and variably impaired autonomy, and one of its important implications—these patients should not be judged uniformly to be without decision-making capacity.

Another implication of chronically and variably impaired autonomy is that strategies of "weak paternalism" cannot be invoked either. Weak paternalism involves an intervention taken to benefit a patient who has fallen below the threshold of autonomous decision-making. Weak paternalism, however, cannot meaningfully be invoked on the basis for intervening in such a patient's decision-making, because there is some substantial, if variable, autonomy that will be seriously violated in the process. Nor can strong paternalism—the violation of the patient's autonomy for his or her good—be justified for the same reason: There is some substantial, if variable, autonomy that will be seriously violated in the process. Some third ethically justified response is called for, and we turn now to the task of identifying such a response.

Ethically justified guidelines

Chronically and variably impaired autonomy, then, means that there are always some one or more aspects of impaired autonomy in patients with chronic mental illness. It is important to recognize that the variable aspects of impaired autonomy may be responsive to ameliorative interventions, because this clinical reality serves as the basis for a series of ethically justified guidelines for family planning interventions to prevent pregnancy in female chronically mentally ill patients. These guidelines thus go beyond the recent guidelines of The American College of Obstetricians and Gynecologists[2] and the literature on mentally retarded patients,[46] which do not take account of the phenomenon of chronically and variably impaired autonomy.

A set of guidelines for patients who can achieve thresholds of autonomy

The first-line response should therefore be to treat chronically and variably impaired autonomy, with the aim of restoring all aspects of impaired autonomous decision-making to a degree at or above a nominal threshold. Each of these aspects, described in the six steps listed, has its own threshold. These thresholds are patient and decision specific. Substantially autonomous decision-making occurs when the patient can exercise all of the capacities at or above their thresholds with respect to the decision at hand (e.g., to use contraceptive barriers).

In some cases patients with chronic mental illness will meet or exceed all of these thresholds. Since clinical judgment about such matters concerns the patient's mental illness and its effects on decision-making capacity, the involvement of a psychiatrist in implementing the guidelines we propose may be very helpful. Securing such involvement in most hospital settings should not be difficult, inasmuch as consultation-liaison psychiatric services are generally available. In other settings, especially family planning centers, appropriate liaison should be established with psychiatric centers, including community mental health programs.[47]

Restoring chronically and variably impaired autonomy involves the following steps:

a. Identify those aspects (steps 1 to 6 as listed previously) of autonomous decision-making that are chronically impaired.
b. Document the history of the variability of impairment, with particular attention to factors that exacerbate that impairment (e.g., psychosocial stressors, drug abuse, going off medication, or medical illness).
c. Identify and implement interventions aimed at improving impaired aspects of autonomous decision-making to a degree at or above the threshold (e.g., medication for loose associations, inten-

sive, repeated provision of information,[48] and removal of psychosocial stressors).

d. Implement the six-step decision-making process described. When the patient's decision-making capacities are at or above their thresholds, respect for the patient's autonomy is meaningful and therefore justified. In such cases the patient is capable of participating in the six-step decision-making process described. In short, such patients are capable of participating in the informed consent process.

A set of guidelines for patients irreversibly near thresholds of autonomy

Sometimes efforts to ameliorate chronically and variably impaired autonomy will not succeed, but the patient is nonetheless near thresholds for substantially autonomous decision-making as a result of the interventions just described. Such patients may meet some thresholds but not others. Simply deciding for such patients what is to be done may impose on the patient values different from those held by the patient, a subtle form of paternalism. Chronically and variably impaired autonomy does not in all cases necessarily imply that such values are unknown or unknowable to the physician. They may have been expressed or utilized by the patient (e.g., in step 4, 5, or 6 of the decision-making process described). The patient's values are therefore, in principle, available as the basis for a response to chronically and variably impaired autonomy irreversibly near but not at thresholds for substantially autonomous decision-making. A patient irreversibly near the thresholds for decision-making can at least assent to medical care and can do so meaningfully if the care plan implemented respects her values.

The strategy of this response is to present alternatives to the patient that are reliably judged to be consistent with her values and ask her to select among them. This second strategy for managing chronically and variably impaired autonomy involves the following:

a. Carefully document the patient's expressed values and beliefs related to reproduction.
b. To further identify the patient's values ask her what is important in her life regarding the decision that needs to be made. For example, what are the patient's feelings about being able to successfully prevent conception, her ability to complete a pregnancy safely, her ability to rear a child?
c. It may be useful to make inquiries of the family's perspective, as well as that of the parents, sexual partner, or spouse, since their insights may be valuable. This should be done only with the agreement of the patient. Family members may also have a vested interest in the outcome, especially when it is the family that has requested family planning intervention. Patients should be protected from undue pressure from family members. When there is undue pressure, the patient should be provided with an opportunity to discuss the issues independently of the family.
d. Identify alternatives (including doing nothing) that are consistent with the patient's values and beliefs.
e. Present these alternatives and ask the patient for her choice among them.

A set of guidelines for patients irreversibly below thresholds of autonomy

Sometimes efforts to bring a patient to or near thresholds for substantially autonomous decision-making fail. Such patients are irreversibly substantially below one or more thresholds. They may therefore be irreversibly below all six thresholds. In the literature, the scope of ethical concern is typically expanded beyond the patient's interests to include the interests of possible future children and social costs.[49, 50] How should each of these three factors shape clinical ethical judgment?

The patient's interests. The interests of the patient concern whether pregnancy can be reliably judged to pose significant mental health and physical benefits and risks to the patient. These risks were discussed at the beginning of this article. The mental health and physical risks discussed may be serious (e.g., disruption of psychiatric treatment or self-destructive behavior), but this is not always the case. Fewer still of these risks are irreversible. Mental health risks brought on by pregnancy can be addressed through rigorous biopsychosocial interventions, as can risks to physical health. It is rarely the case that those risks are predictable with reliability, even in patients with documented history of the occurrence of such risks in a prior pregnancy.

The justification for weak paternalism rests on beneficence-based grounds, namely, to avoid predictable irreversible and serious harm to the patient. If this interpretation of the risks of pregnancy for patients with chronic mental illness is correct, these grounds are, as a rule, not satisfied. Thus a paternalistic response to patients without decision-making capacity (i.e., to simply make decisions for them) is not ethically justified.

Risks to possible future children. The risks to possible future children involve the ethical issues of harm to others than the patient. Those risks involve both genetic and social risks. There are some circumstances in which a possible future child is at significant risk for genetic mental illness (e.g., schizophrenia). Schizophrenia is certainly a serious disease, and there is some intuitive appeal to saying the children born with schizophrenia to two parents with the disease might well be better off having never existed. This intuition, in our view, rests on a contended concept, namely, wrongful life. This is the concept that life under certain circum-

stances is worse than being dead.[51] The problem here is that being dead is not a benefit to someone, because there is no "someone" to be benefited. Thus this intuition fails to be conceptually well grounded.

There are social risks to the children of one or two parents with chronic mental illness. These range along a continuum from disrupted or even impaired development to court-ordered removal from the home. These risks may be reversible over time and unpredictable in many cases. Thus serious irreversible social risks to such children are difficult to predict with certainty or even high probability. If the preceding analysis is correct, making reproductive decisions for these patients cannot be justified on the basis of avoiding harm to possible future children.

Social costs. Social costs, however, of pregnancy, birth, and child rearing by women with chronic mental illness do exist. The ethical issue here is whether society can reasonably invoke a justice-based claim to avoid such costs. Society has declined to do so thus far. There is a good reason for this policy. These women are not the only ones at risk for incurring social costs to society from their reproductive decisions. Teenage women and men, for example, constitute a group whose reproductive decisions cause considerable social costs, yet no attempt is made to restrict their reproductive decisions. Picking out only women with chronic mental illness for restriction of reproductive decisions because of social costs would therefore constitute arbitrary discrimination and would be patently unjust. Picking out all women whose reproductive decisions involve social costs would constitute wholesale violation of their constitutional and civil rights, the right to privacy in particular, which is also patently unjust. Thus appeals to avoiding harm to society cannot be justified.

The social costs to parents, spouses, and sexual partners of patients with chronic mental illness may be legitimate considerations. For example, the strong preference of parents who bring the patient for treatment not to be responsible for rearing a grandchild are understandable. However, harm to these legitimate interests is not inevitable, inasmuch as other alternatives are available (e.g., should she become pregnant, the woman raises her child by herself or places her child for adoption). The strong preference of a husband or sexual partner to avoid a pregnancy for the patient can be readily satisfied by his undertaking appropriate measures to ensure his own sterility. Thus harm to his legitimate interests is not inevitable either.

When appeals to paternalism and harm to others cannot succeed, there is no ethical justification for overriding contraceptive choices. The second set of guidelines presented here should therefore be followed, with two considerations.

First, it is not justified to force surgical sterilization on patients whose autonomy is irreversibly lost or to coerce or manipulate them into accepting surgical sterilization, especially since there is now an effective, long-term, implantable, reversible contraceptive device (Norplant).[52]

Second, contraceptive interventions need to be effective, given the consequences of unwanted pregnancies in this population of patients. Because of compliance problems with oral contraceptives,[3] this method of contraception might not be effective in this population. Barrier techniques will be even less effective. By contrast, the Norplant device is significantly more likely to be effective.[52] It has the additional advantage of less serious risks of morbidity associated with long-term use.

The Norplant device must be implanted and removed by a surgical procedure. This factor is ethically significant, because of the potential for abuse. That abuse would take the form of the physician refusing a request by a chronically mentally ill patient to insert or to remove it. Because, as we have shown, appeals to paternalism or harm to others cannot succeed, there is no ethical justification for refusing the patient's request to remove a Norplant device.

In conclusion, patients with chronic mental illness constitute a particularly vulnerable group of patients, with respect to access to gynecologic care and to dehumanization. At the same time such patients constitute an ethically and clinically unique group in terms of autonomy. The proposed guidelines respect the autonomy of these patients and we believe, if adopted, would serve as powerful antidotes to both of these problems and thus contribute significantly to the quality of obstetric and gynecologic care for female patients with chronic mental illness.

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A prospective, controlled multicenter study on the obstetric risks of pregnant women with antiphospholipid antibodies

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OBJECTIVES: A prospective, controlled multicenter study was performed to estimate the obstetric risks of antiphospholipid antibodies (the lupus anticoagulant and anticardiolipin antibodies). In addition, the risks of prior thrombosis, obstetric history, systemic lupus erythematosus, and high-dose prednisone treatment were evaluated.

STUDY DESIGN: After screening for antiphospholipid antibodies in patients with lupus erythematosus or women with prior fetal loss(es), 59 subsequent pregnancies with and 54 without these antibodies were followed.

RESULTS: The presence of the lupus anticoagulant and a history of at least three spontaneous abortions could predict fetal loss ($p = 0.032$ and 0.001, respectively). In live born infants, a low birth weight could be predicted by the presence of anticardiolipin antibodies ($p = 0.034$), prior intrauterine fetal death ($p = 0.025$), and treatment with high-dose prednisone ($p = 0.002$). No relationships were seen between antiphospholipid antibodies and small-for-gestational-age newborns and pregnancy-induced hypertension or preeclampsia. The disappearance of antiphospholipid antibodies during pregnancy was not correlated with live birth.

CONCLUSION: It is concluded that the presence of antiphospholipid antibodies is a risk factor for adverse pregnancy outcome. (Am J Obstet Gynecol 1992;167:26-32.)

Key words: Lupus anticoagulant, anticardiolipin antibodies, pregnancy outcome, systemic lupus erythematosus

In the last decade antiphospholipid antibodies, notably the lupus anticoagulant and anticardiolipin antibodies, have received much attention. Their presence has been associated with prior fetal loss, thrombosis, and thrombocytopenia in retrospective reports.[1-3] These correlations have been described in patients with systemic lupus erythematosus, lupuslike disease, and fetal loss without underlying disease.[4, 5] In patients with antiphospholipid antibodies, fetal loss is generally accompanied by placental thrombosis. However, the pathogenetic potential of these antibodies has not been demonstrated.[6]

There are no large-scale studies evaluating the obstetric risks for pregnant women with antiphospholipid antibodies in a prospective, controlled study. However, on the basis of studies on small series or case reports, it has become current practice to offer some kind of treatment. Successes have been claimed in favor of a therapy that includes high-dose prednisone,[7, 8] but its benefits are also controversial.[9] Other successful treatments reported include subcutaneous heparin or low-dose aspirin.[9, 10]

The need for large prospective, controlled studies in pregnant women with antiphospholipid antibodies is universally recognized.[11, 12] In this study we prospectively evaluated the risks of lupus anticoagulant and anticardiolipin antibodies (both level and isotype) on pregnancy outcome in 59 pregnancies complicated by antiphospholipid antibodies compared with 54 control pregnancies. We also estimated the obstetric risks of systemic lupus erythematosus, lupuslike disease, prior adverse pregnancy outcomes, history of thrombosis, and inclusion of high-dose prednisone in the treatment regimen.

Material and methods

Patients. From June 1987 women who wished to conceive and who were from risk groups were screened for the presence of antiphospholipid antibodies to identify eligible patients for this study. Patients were obtained from all over the country. Risk groups included patients with systemic lupus erythematosus according

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to American Rheumatism Association-criteria[13]; patients with lupuslike disease (fewer than four criteria); and patients with at least three unexplained spontaneous abortions before 12 weeks of gestation or at least one intrauterine fetal death after 12 weeks not caused by infection, abruptio placentae, apparent congenital malformations, or chromosomal abnormalities. In all patients with at least three spontaneous abortions hysterosalpingography was performed to exclude uterine malformations. A total of 264 patients were screened, including 61 with systemic lupus erythematosus, 33 with lupuslike disease, and 170 with prior adverse pregnancy outcomes. Antiphospholipid antibodies were found in 115 patients (44%), including 29 with both lupus anticoagulant and anticardiolipin antibodies (25%), one with lupus anticoagulant only (1%), and 85 with anticardiolipin antibodies only (74%, see *Samples and assays*). Fifty-two of 115 patients with antiphospholipid antibodies (45%) subsequently had 59 pregnancies (group A). Of these, 19 were characterized by both lupus anticoagulant and anticardiolipin antibodies, one by lupus anticoagulant only, and 39 by anticardiolipin antibodies only. Of 149 women without antiphospholipid antibodies after screening, 54 had a subsequent pregnancy (36%, group B). In group A, 10 patients had experienced a prior thrombosis (19%) compared with four patients in group B (7%).

Treatment. Treatment, including high-dose prednisone (≥40 mg), was given in 11 patients. Of these, four received prednisone alone, three received prednisone plus low-dose aspirin (80 mg), and four received prednisone plus 2 × 5000 IU regular heparin subcutaneously. All but one had medium- or high-positive levels of anticardiolipin antibodies or lupus anticoagulant. Two patients with systemic lupus erythematosus in remission received prednisone for earlier activity and one as treatment of a lupus exacerbation. In all other patients treatment was given prophylactically because of the presence of antiphospholipid antibodies. Other treatments in group A included 80 mg aspirin in 16 and 2 × 5000 IU heparin given subcutaneously in three patients. In 29 pregnancies from group A no treatment was given. Treatment was given as soon as pregnancy was confirmed by ultrasonography and was continued at the same dose until 6 weeks postpartum. In patients using heparin, oral anticoagulants were given between 16 and 36 weeks of gestation instead of the heparin. In patients with medium or high levels of anticardiolipin antibodies or lupus anticoagulant allocation of treatment between 40 mg prednisone plus 80 mg aspirin versus aspirin alone, or between 40 mg prednisone plus 2 × 5000 IU subcutaneous regular heparin versus heparin alone in patients receiving oral anticoagulants because of prior thrombosis, was initially intended after randomization. However, because 28 of the 32 pregnant women eligible for randomization were reluctant to use high-dose prednisone because of potential side effects, only four were randomized.

Samples and assays

Samples. In addition to the samples obtained for initial screening, monthly blood samples from 19 of 20 patients with lupus anticoagulant and 22 of 31 patients with medium- or high-positive levels of anticardiolipin antibodies after screening were collected during pregnancy and in the postpartum period (median eight samples, range 1 to 15). Samples from patients seen in hospitals other than in University Hospital of Utrecht were sent by mail and stored at −70° C within 24 hours. All assays were performed in this hospital.

Blood samples for the determination of lupus anticoagulant were taken by venipuncture into a 0.1 volume of 3.8% trisodium citrate. Platelet-poor plasma was prepared by centrifuging the blood twice at 4° C at 2000*g* for 15 minutes. Blood samples for the preparation of serum for determinations of anticardiolipin antibodies were collected by venipuncture into glass tubes. The blood was allowed to clot at room temperature for 2 hours and centrifuged at 2000*g* for 10 minutes to obtain serum.

Lupus anticoagulant. Three lupus anticoagulant assays were done as described previously[14]: (1) a partial thromboplastin time with a 1 : 1 mixture of patient and pooled normal plasma with thromboplastin derived from human brain, (2) the partial thromboplastin time of test plasma on dilution of thromboplastin, and (3) kaolin clotting time index, calculated from the kaolin clotting time of control and test plasma, mixed at different ratios. Lupus anticoagulant was considered to be present when at least two assays were positive.

Anticardiolipin antibodies. Anticardiolipin antibodies immunoglobulin G and immunoglobulin M were measured by enzyme-linked immunosorbent assay as described earlier[14] in a 1 : 100 serum dilution. The Kingston Antiphospholipid Antibody Study Group standards were included on all enzyme-linked immunosorbent assay plates to establish cutoff levels between high-, medium-, and low-positive and normal levels, according to the second anticardiolipin standardization exercise.[15]

End points. The end points of this study were fetal death, birth weight, the occurrence of small-for-gestational-age (SGA) newborns with a birth weight below the 10 percentile according to Kloosterman curves corrected for sex and parity,[16] pregnancy-induced hypertension defined as a diastolic pressure of ≥90 mm Hg after the twenty-fourth week of pregnancy without preexisting hypertension, and preeclampsia defined as pregnancy-induced hypertension plus proteinuria of ≥0.5 gm/L.[17] Active lupus nephritis was regarded to be present when, apart from proteinuria or an elevated

Table I. Patient characteristics and pregnancy outcome in 59 pregnancies of patients with antiphospholipid antibodies (group A) and 54 pregnancies of patients without these antibodies (group B)

	Group A	*Group B*
Patients (No.)	52	54
No. of pregnancies	59	54
Systemic lupus erythematosus (n = 37)	29	8
Lupuslike disease (n = 12)	7	5
Prior adverse pregnancy outcome (n = 64)	23	41
At least 3 spontaneous abortions	11	13
At least 1 intrauterine fetal death	9	14
Both spontaneous abortions and intrauterine fetal death	3	14
Outcome		
Spontaneous abortions (n = 15)	7 (12%)	8 (15%)
Intrauterine fetal death (n = 10)	7 (12%)	3 (6%)
Live infant (n = 88)	45 (76%)	43 (79%)
Gestational age* (wk, mean ± SD)	36.9 ± 3.6	38.7 ± 1.6
Birth weight* (gm, mean ± SD)	2665 ± 945	3090 ± 605
No. of children <10th percentile*	11 (24%)	9 (21%)
No. of pregnancies <37 wk*	21 (36%)	3 (7%)
No. of pregnancies with pregnancy-induced hypertension or preeclampsia	10 (22%)	10 (23%)
No. of cesarean sections because of fetal distress	14 (31%)	5 (12%)
Mean gestational age (wk)	33.5	36.6

Fetal loss was predicted by lupus anticoagulant (p = 0.032) and at least three spontaneous abortions in the past (p = 0.001). Low birth weight was significantly associated with a history of intrauterine fetal death (p = 0.025), presence of anticardiolipin antibodies (p = 0.034), and treatment with high-dose prednisone (p = 0.002). Statistical analysis was performed with stepwise multiple and logistic regression.

*Of live infants.

serum creatinine level, extrarenal symptoms suggestive of active disease or an active urine sediment were present.

Statistics. Statistical analysis was performed with Fisher's exact test and stepwise multiple and logistic regression with the statistical package SPSS/PC+ version 3.1 (SPSS, Chicago) and BMDP, version 1987 (BMDP, University of California, Berkeley), to determine the risks of lupus anticoagulant and anticardiolipin antibodies (both level and isotype), systemic lupus erythematosus, lupuslike disease, at least three spontaneous abortions in the past, at least one prior intrauterine fetal death, high-dose prednisone treatment, and a history of thrombosis on the end points, considering all pregnancies (n = 113) as a whole. This technique was chosen because it accounts for the influence of overlaps between variables.

Results

Pregnancy outcome

Fetal loss. In group A, seven spontaneous abortions (12%) and seven intrauterine fetal deaths (12%) occurred versus eight abortions (15%) and three stillbirths (6%) in group B (Table I). Fetal loss was predicted by the presence of lupus anticoagulant (p = 0.032) or a history of at least three spontaneous abortions (p = 0.001) and not by anticardiolipin antibody level or isotype, a history of thrombosis, or the presence of systemic lupus erythematosus or lupuslike disease. The 20 pregnancies with lupus anticoagulant ended in three spontaneous abortions (15%) and four intrauterine fetal deaths (20%), and the 33 pregnancies of patients with at least three spontaneous abortions in the past resulted in 10 spontaneous abortions (30%) and four intrauterine fetal deaths (12%, Fig. 1). All placentas from lupus anticoagulant–positive pregnancies ending in intrauterine fetal death demonstrated signs of thrombosis or infarction. In the placentas from the three stillbirths in group B, no signs of thrombosis or infarction were found. Of 58 patients from group A with anticardiolipin antibodies, seven abortions (12%) and 7 intrauterine fetal deaths (12%) occurred. In the 11 patients treated with high-dose prednisone, one spontaneous abortion (9%) and three intrauterine fetal deaths (27%) occurred, versus two spontaneous abortions (11%) and two intrauterine fetal deaths (11%) in the 19 patients treated with low-dose aspirin or heparin (p = 0.310, Table II). In the 29 patients from group A without any treatment, four spontaneous abortions (14%) and two intrauterine fetal deaths occurred (7%, Table II).

Birth weight and gestational age of live infants. The relationship between birth weight and gestational age at delivery of live infants is shown in Fig. 2. Mean birth weight and gestational age were 2665 gm and 36.9 weeks in group A and 3090 gm and 38.7 weeks in group B. Birth weight was significantly lower in patients with anticardiolipin antibodies (mean weight 2660 gm, p = 0.034), prior intrauterine fetal death (mean weight of 2765 gm after one stillbirth, 2610 gm after two or more, p = 0.025) and in patients treated with high-dose prednisone (mean weight 1870 gm, p = 0.002) compared with patients without these characteristics. Mean birth weight and gestational age in patients treated with low-dose aspirin or heparin were 2565 gm and 36.5 weeks (Table II).

Twenty-one pregnancies (36%) in group A and three

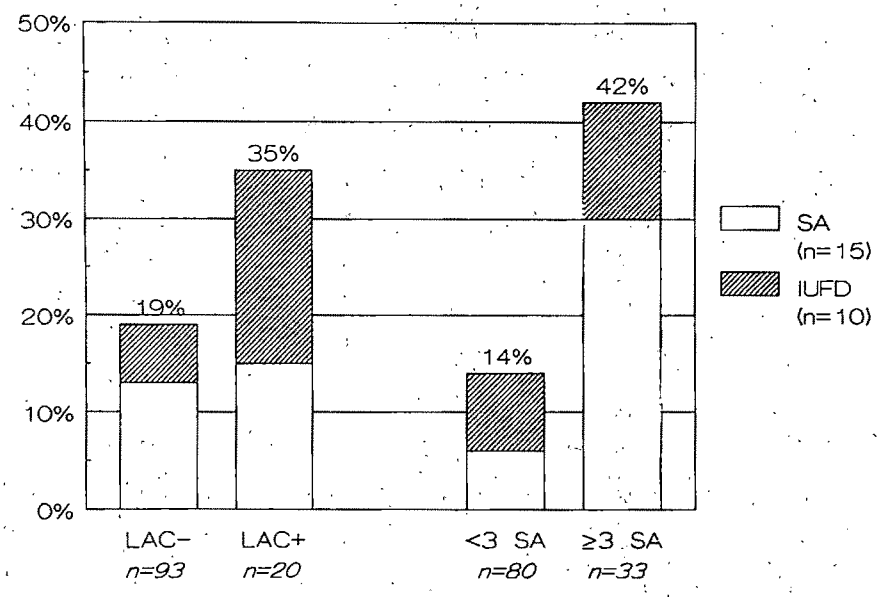


Fig. 1. Fetal loss rates in women with (n = 20) or without (n = 93) lupus anticoagulant and in women with at least three prior spontaneous abortions (*SA*, n = 33) or less (n = 80). Lupus anticoagulant and history of at least three spontaneous abortions predicted fetal loss (p = 0.032 and p = 0.001, respectively). Note that increased fetal loss rate in women with lupus anticoagulant is due to intrauterine fetal death *(IUFD)* after 12 weeks of gestational age, in contrast to increased fetal loss rate in women with at least three previous spontaneous abortions, who primarily aborted in first 12 weeks of pregnancy.

(7%) in group B ended before 37 weeks of gestation ($p < 0.001$). Fourteen cesarean sections (31%) in group A and five (12%) in group B were performed because of fetal distress (p = 0.032). The mean gestational age at time of cesarean section was 33.6 weeks in group A and 36.6 weeks in group B.

SGA. Eleven children in group A (24%) and nine in group B (21%) were SGA, which could not be predicted by any of the variables.

Pregnancy-induced hypertension or preeclampsia. Ten patients in group A (22%) had pregnancy-induced hypertension or preeclampsia versus 10 in group B (23%). None of the evaluated variables could predict these complications. None of the lupus patients developed lupus nephritis.

Complications of treatment. In the 11 patients treated with high-dose prednisone, a cushingoid face was present in all, severe striae in one, and acne in four. No vertebral collapses occurred. In one patient treated with subcutaneous heparin an abscess developed at the injection site. No side effects of low-dose aspirin were seen nor were any drug-related neonatal complications.

Follow-up of antiphospholipid antibodies during pregnancy. From 19 evaluated lupus anticoagulant–positive pregnancies, lupus anticoagulant disappeared during pregnancy in eight patients with two fetal losses. In 11 pregnancies, with persistently present lupus anticoagulant, three fetal deaths occurred.

From 22 evaluated pregnancies with medium- or high-positive lupus anticoagulant anticardiolipin antibody levels after screening, three patients reached low or negative levels without fetal losses. Fourteen live births and five fetal losses occurred in 19 pregnancies with persistently medium- or high-positive anticardiolipin antibody levels. There was no significant association between the disappearance of lupus anticoagulant (p = 0.664) or anticardiolipin antibodies (p = 0.441) and live birth.

Comment

This is the first prospective controlled study that evaluated the obstetric risk of antiphospholipid antibodies. With a statistical analysis technique accounting for the influence of overlap between the evaluated variables, only lupus anticoagulant and the occurrence of at least three spontaneous abortions in the past predicted fetal loss, in contrast to anticardiolipin antibody isotype or level, systemic lupus erythematosus, or lupus-like disease and a history of thrombosis. In the patients with antiphospholipid antibodies, spontaneous abortion rates (12% for anticardiolipin antibody and 15% for lupus anticoagulant) were comparable with the normal population. However, in patients with lupus anticoagulant most fetal losses were after 12 weeks of gestation (four of seven losses in 20 pregnancies), whereas patients with a history of spontaneous abortions mainly had early abortions (10 of 14 losses in 33 pregnancies, Fig. 1). An increasing chance for a spontaneous abor-

Table II. Pregnancy outcome in relation to treatment in 59 pregnancies characterized by the presence of antiphospholipid antibodies

	Treatment including high-dose prednisone (n = 11)	*Treatment with low-dose aspirin (n = 16) or heparin (n = 3)*	*No treatment (n = 29)*
No. of pregnancies			
Systemic lupus erythematosus (n = 29)	10	7	12
Lupuslike disease (n = 7)	1	4	2
Prior adverse pregnancy outcome (n = 23)	0	8	15
Lupus anticoagulant–positive (n = 20)	8	11	1
Lupus anticoagulant–negative plus low-positive anticardiolipin antibody levels (n = 27)	1	5	21
Lupus anticoagulant–negative plus medium- or high-positive anticardiolipin antibody levels (n = 12)	2	3	7
Outcome			
Spontaneous abortion (n = 7)	1 (9%)	2 (11%)	4 (14%)
Intrauterine fetal death (n = 7)	3 (27%)	2 (11%)	2 (7%)
Alive (n = 45)	7 (64%)	15 (78%)	23 (79%)
Gestational age* (wk, mean ± SD)	33.7 ± 2.0	36.5 ± 4.1	38.2 ± 2.9
Birth weight* (gm, mean ± SD)	1870 ± 850	2565 ± 1005	2972 ± 805
No. of children <10th percentile*	2 (29%)	3 (20%)	6 (26%)
No. of pregnancies with pregnancy-induced hypertension or preeclampsia	2 (29%)	5 (33%)	3 (10%)
No. of cesarean sections because of fetal distress	4 (57%)	5 (33%)	5 (17%)

Treatment containing high-dose prednisone was significantly associated with low birth weight ($p = 0.002$), as analyzed with stepwise multiple regression.

*Of live infants.

tion after an unsuccessful past reproductive performance has been described earlier.[18] The cause of lupus anticoagulant–associated intrauterine fetal death is probably placental insufficiency, because signs of thrombosis or infarction were present in all placentas. This is in accordance with a recently published study of ours in which we demonstrated that intrauterine fetal death in patients with antiphospholipid antibodies is significantly associated with histopathologic features of thrombosis or infarction, compared with a control group of women with fetal loss without these antibodies.[19] We did not find a relation between anticardiolipin antibodies and fetal loss. This may be due to the antiphospholipid antibody–negative control group, which is not comparable with the normal population. We chose this control group because possible risk factors for adverse pregnancy outcome frequently found in women with antiphospholipid antibodies (systemic lupus erythematosus, prior fetal losses) were present in these patients. Use of this group made optimal discrimination between the impact of the various risk factors on pregnancy outcome possible. Therefore it is likely that the significant relations we found will be even more apparent when compared with the normal population. The composition of the control group may also be responsible for the fact that we did not find a significant relation between antiphospholipid antibodies and SGA newborns or pregnancy-induced hypertension or preeclampsia. Both conditions had a higher incidence in our control group than in a normal population. In the Netherlands SGA newborns are seen by definition in 10% of all pregnancies. Normal mean birth weight is 3445 gm.[16]

We found a 44% prevalence of antiphospholipid antibodies in our screening population, which included not only patients with bad obstetric histories but also patients with systemic lupus erythematosus and lupuslike disease referred to our center because of obstetric problems.

In live infants a low birth weight could be predicted independently by the presence of anticardiolipin antibodies, prior intrauterine fetal death, and treatment with high-dose prednisone. Birth before 37 weeks of gestation was seen significantly more often in pregnancies with antiphospholipid antibodies. This was in part artificial because of the significantly increased cesarean section rate associated with fetal distress. It further illustrates the poor fetal condition in pregnancies with antiphospholipid antibodies, leading to early obstetric intervention.

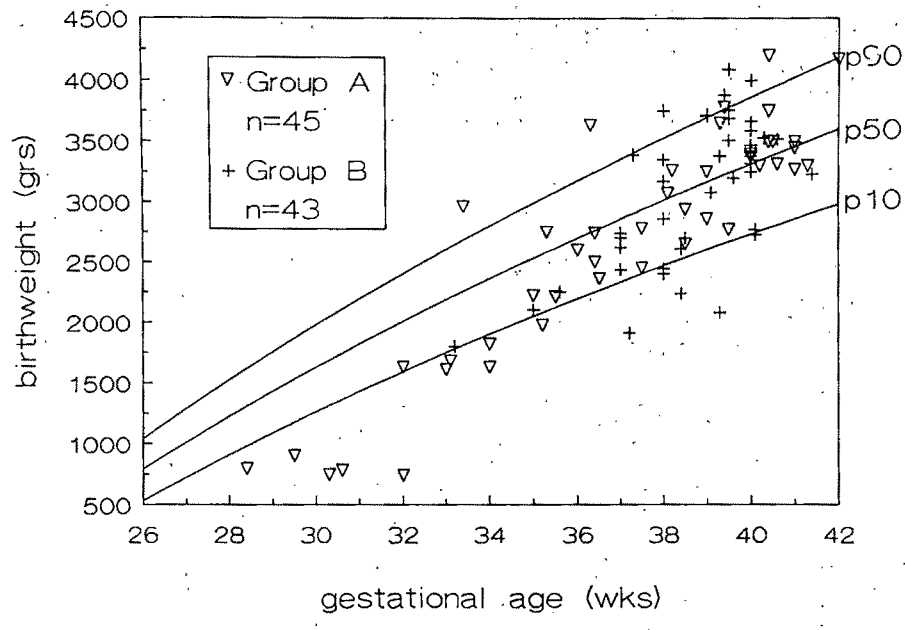


Fig. 2. Relationship of birth weight and gestational age of live infants, including 45 pregnancies with (group A) and 43 pregnancies without (group B) antiphospholipid antibodies compared with normal percentiles *(p10, p50, p90)*.

The initially intended randomization procedure failed due to patient reluctance to use high-dose prednisone. Besides, we demonstrated a significantly lower birth weight after treatment with high-dose prednisone during pregnancy. These are important observations in view of possible future randomized trials on this subject. We also could not demonstrate a superior outcome after prednisone therapy, which is in agreement with findings of Lockshin et al.,[9] who even found fetal loss in nine of 11 patients (84%) with high-positive anticardiolipin antibodies and prior fetal death. Our somewhat better results after prednisone treatment may be explained by the fact that our series included three patients without a prior fetal death and three patients with only low- or medium-positive anticardiolipin antibody levels in the absence of lupus anticoagulant. Considering these disappointing effects after steroid treatment, we believe that high-dose prednisone therapy is not the first choice in any forthcoming trial or in the management of individual patients. Unfortunately, our study does not allow firm conclusions on what treatment should be given instead, because of its limited size and nonrandomized nature.

Although it is often claimed that complete suppression of the antiphospholipid antibodies is mandatory for a favorable outcome,[7, 20] we could not confirm this concept. Therefore the use of high-dose prednisone for this purpose does not seem to be indicated. Instead, antithrombotic treatment with low-dose aspirin or heparin may be sufficient in preventing fetal loss, in spite of the prolonged presence of antiphospholipid antibodies. However, it was remarkable to notice that in the 29 patients with antiphospholipid antibodies who were not treated at all fetal loss occurred in 21%, compared with 22% in patients who received mild therapy with low-dose aspirin or heparin. The necessity of any treatment therefore remains to be proved. In conclusion, our prospective, controlled data indicate that lupus anticoagulant should be considered a risk factor for fetal loss and anticardiolipin antibodies for a low birth weight. Therefore pregnancies in women with antiphospholipid antibodies are high-risk pregnancies, demanding close fetal monitoring especially when a history of fetal loss is present. High-dose prednisone is not the first choice of treatment in these patients. Future randomized studies have to be done to establish whether close fetal surveillance is sufficient for a successful pregnancy or whether any antithrombotic drug treatment should be added for optimal outcome.

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BRIEF SUMMARY

Tri-Levlen®—6 brown tablets, each containing 0.050 mg of levonorgestrel (d(-)-13 beta-ethyl-17-alpha-ethinyl-17-beta-hydroxygon-4-en-3-one), a totally synthetic progestogen, and 0.030 mg of ethinyl estradiol (19-nor-17α-pregna-1,3,5(10)-trien-20-yne-3, 17-diol); 5 white tablets, each containing 0.075 mg levonorgestrel and 0.040 mg ethinyl estradiol; 10 light-yellow tablets, each containing 0.125 mg levonorgestrel and 0.030 mg ethinyl estradiol (7 light-green tablets containing inert ingredients are included in the 28-day triphasic regimen).

Indications and Usage

Oral contraceptives are indicated for the prevention of pregnancy in women who elect to use this product as a method of contraception.

Contraindications

Oral contraceptives should not be used in women with any of the following conditions: thrombophlebitis or thromboembolic disorders, a past history of deep-vein thrombophlebitis or thromboembolic disorders, cerebral-vascular or coronary-artery disease, known or suspected carcinoma of the breast, carcinoma of the endometrium or other known or suspected estrogen-dependant neoplasia, undiagnosed abnormal genital bleeding, cholestatic jaundice of pregnancy or jaundice with prior pill use, hepatic adenomas or carcinomas, known or suspected pregnancy.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral-contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risks of several serious conditions including myocardial infarction, thromboembolism, stroke, hepatic neoplasia, gallbladder disease, and hypertension, although the risk of serious morbidity or mortality is very small in healthy women without underlying risk factors. The risk of morbidity and mortality increases significantly in the presence of other underlying risk factors such as hypertension, hyperlipidemias, obesity and diabetes. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks. The information contained in this package insert is principally based on studies carried out in patients who used oral contraceptives with higher formulations of estrogens and progestogens than those in common use today. The effect of long-term use of the oral contraceptives with lower formulations of both estrogens and progestogens remains to be determined. Throughout this labeling, epidemiological studies reported are of two types: retrospective or case control studies and prospective or cohort studies. Case control studies provide a measure of the relative risk of disease, namely, a ratio of the incidence of a disease among oral-contraceptive users to that among nonusers. The relative risk does not provide information on the actual clinical occurrence of a disease. Cohort studies provide a measure of attributable risk, which is the difference in the incidence of disease between oral-contraceptive users and nonusers. The attributable risk does provide information about the actual occurrence of a disease in the population. For further information, the reader is referred to a text on epidemiological methods.

1. *Thromboembolic Disorders and Other Vascular Problems*

a. *Myocardial Infarction.* An increased risk of myocardial infarction has been attributed to oral-contraceptive use. This risk is primarily in smokers or women with other underlying risk factors for coronary-artery disease such as hypertension, hypercholesterolemia, morbid obesity, and diabetes. The relative risk of heart attack for current oral-contraceptive users has been estimated to be two to six. The risk is very low under the age of 30. Smoking in combination with oral-contraceptive use has been shown to contribute substantially to the incidence of myocardial infarctions in women in their mid-thirties or older with smoking accounting for the majority of excess cases. Mortality rates associated with circulatory disease have been shown to increase substantially in smokers over the age of 35 and non-smokers over the age of 40 among women who use oral contraceptives. Oral contraceptives may compound the effects of well-known risk factors, such as hypertension, diabetes, hyperlipidemias, age and obesity. In particular, some progestogens are known to decrease HDL cholesterol and cause glucose intolerance, while estrogens may create a state of hyperinsulinism. Oral contraceptives have been shown to increase blood pressure among users (see section 9 in "Warnings"). Similar effects on risk factors have been associated with an increased risk of heart disease. Oral contraceptives must be used with caution in women with cardiovascular disease risk factors.

b. *Thromboembolism.* An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Case control studies have found the relative risk of users compared to nonusers to be 3 for the first episode of superficial venous thrombosis, 4 to 11 for deep-vein thrombosis or pulmonary embolism, and 1.5 to 6 for women with predisposing conditions for venous thromboembolic disease. Cohort studies have shown the relative risk to be somewhat lower, about 3 for new cases and about 4.5 for new cases requiring hospitalization. The risk of thromboembolic disease due to oral contraceptives is not related to length of use and disappears after pill use is stopped. A two-to four-fold increase in relative risk of postoperative thromboembolic complications has been reported with the use of oral contraceptives. The relative risk of venous thrombosis in women who have predisposing conditions is twice that of women without such medical conditions. If feasible, oral contraceptives should be discontinued at least four weeks prior to and for two weeks after elective surgery of a type associated with an increase in risk of thromboembolism and during and following prolonged immobilization. Since the immediate post partum period is also associated with an increased risk of thromboembolism, oral contraceptives should be started no earlier than four to six weeks after delivery in women who elect not to breast-feed, or a midtrimester pregnancy termination.

c. *Cerebrovascular diseases.* Oral contraceptives have been shown to increase both the relative and attributable risks of cerebrovascular events (thrombotic and hemorrhagic strokes), although, in general, the risk is greatest among older (>35 years), hypertensive women who also smoke. Hypertension was found to be a risk factor for both users and nonusers, for both types of strokes, while smoking interacted to increase the risk for hemorrhagic strokes. In a large study, the relative risk of thrombotic strokes has been shown to range from 3 for normotensive users to 14 for users with severe hypertension. The relative risk of hemorrhagic stroke is reported to be 1.2 for nonsmokers who used oral contraceptives, 2.6 for smokers who did not use oral contraceptives, 7.6 for smokers who used oral contraceptives, 1.8 for normotensive users and 25.7 for users with severe hypertension. The attributable risk is also greater in older women.

d. *Dose-related risk of vascular disease from oral contraceptives.* A positive association has been observed between the amount of estrogen and progestogen in oral contraceptives and the risk of vascular disease. A decline in serum high-density lipoproteins (HDL) has been reported with many progestational agents. A decline in serum high-density lipoproteins has been associated with an increased incidence of ischemic heart disease. Because estrogens increase HDL cholesterol, the net effect of an oral contraceptive depends on a balance achieved between doses of estrogen and progestogen and the nature and absolute amount of progestogen used in the contraceptive. The amount of both hormones should be considered in the choice of an oral contraceptive. Minimizing exposure to estrogen and progestogen is in keeping with good principles of therapeutics. For any particular estrogen/progestogen combination, the dosage regimen prescribed should be one which contains the least amount of estrogen and progestogen that is compatible with a low failure rate and the needs of the individual patient. New acceptors of oral-contraceptive agents should be started on preparations containing less than 50 mcg of estrogen.

e. *Persistence of risk of vascular disease.* There are two studies which have shown persistence of risk of vascular disease for ever-users of oral contraceptives. In a study in the United States, the risk of developing myocardial infarction after discontinuing oral contraceptives persists for at least 9 years for women 40-49 years who had used oral contraceptives for five or more years, but this increased risk was not demonstrated in other age groups. In another study in Great Britain, the risk of developing cerebrovascular disease persisted for at least 6 years after discontinuation of oral contraceptives, although excess risk was very small. However, both studies were performed with oral-contraceptive formulations containing 50 micrograms or higher of estrogens.

2. *Estimates of Mortality from Contraceptive Use.* One study gathered data from a variety of sources which have estimated the mortality rate associated with different methods of contraception at different ages. These estimates include the combined risk of death associated with contraceptive methods plus the risk attributable to pregnancy in the event of method failure. Each method of contraception has its specific benefits and risks. The study concluded that with the exception of oral-contraceptive users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of birth control is less than that associated with childbirth. The observation of a possible increase in risk of mortality with age for oral-contraceptive users is based on data gathered in the 1970's—but not reported until 1983. However, current clinical practice involves the use of lower estrogen dose formulations combined with careful restriction of oral-contraceptive use to women who do not have the various risk factors listed in this labeling. Because of these changes in practice and, also, because of some limited new data which suggest that the risk of cardiovascular disease with the use of oral contraceptives may now be less than previously observed, the Fertility and Maternal Health Drugs Advisory Committee was asked to review the topic in 1989. The Committee concluded that although cardiovascular-disease risks may be increased with oral-contraceptive use after age 40 in healthy nonsmoking women (even with the newer low-dose formulations), there are greater potential health risks associated with pregnancy in older women and with the alternative surgical and medical procedures which may be necessary if such women do not have access to effective and acceptable means of contraception. Therefore, the Committee recommended that the benefits of oral-contraceptive use by healthy nonsmoking women over 40 may outweigh the possible risks. Of course, older women, as all women who take oral contraceptives, should take the lowest possible dose formulation that is effective.

3. *Carcinoma of the Reproductive Organs.* Numerous epidemiological studies have been performed on the incidence of breast, endometrial, ovarian and cervical cancer in women using oral contraceptives. The overwhelming evidence in the literature suggests that use of oral contraceptives is not associated with an increase in the risk of developing breast cancer, regardless of the age and parity of first use or with most of the marketed brands and doses. The Cancer and Steroid Hormone (CASH) study also showed no latent effect on the risk of breast cancer for at least a decade following long-term use. A few studies have shown a slightly increased relative risk of developing breast cancer, although the methodology of these studies, which included differences in examination of users and nonusers and differences in age at start of use, has been questioned. Some studies suggest that oral-contraceptive use has been associated with an increase in the risk of cervical intraepithelial neoplasia in some populations of women. However, there continues to be controversy about the extent to which such findings may be due to differences in sexual behavior and other factors. In spite of many studies of the relationship between oral-contraceptive use and breast and cervical cancers, a cause-and-effect relationship has not been established.

4. *Hepatic Neoplasia.* Benign hepatic adenomas are associated with oral-contraceptive use, although the incidence of benign tumors is rare in the United States. Indirect calculations have estimated the attributable risk to be in the range of 3.3 cases/100,000 for users, a risk that increases after four or more years of use. Rupture of rare, benign, hepatic adenomas may cause death through intra-abdominal hemorrhage. Studies from Britain have shown an increased risk of developing hepatocellular carcinoma in long-term (>8 years) oral-contraceptive users. However, these cancers are extremely rare in the U.S. and the attributable risk (the excess incidence) of liver cancers in oral-contraceptive users approaches less than one per million users.

5. *Ocular Lesions.* There have been clinical case reports of retinal thrombosis associated with the use of oral contraceptives. Oral contraceptives should be discontinued if there is unexplained partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions. Appropriate diagnostic and therapeutic measures should be undertaken immediately.

6. *Oral-Contraceptive Use Before or During Early Pregnancy.* Extensive epidemiological studies have revealed no increased risk of birth defects in women who have used oral contraceptives prior to pregnancy. Studies also do not suggest a teratogenic effect, particularly insofar as cardiac anomalies and limb-reduction defects are concerned, when taken inadvertently during early pregnancy. The administration of oral contraceptives to induce withdrawal bleeding should not be used as a test for pregnancy. Oral contraceptives should not be used during pregnancy to treat threatened or habitual abortion. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing oral-contraceptive use. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period. Oral-contraceptive use should be discontinued if pregnancy is confirmed.

7. *Gallbladder Disease.* Earlier studies have reported an increased lifetime relative risk of gallbladder surgery in users of oral contraceptives and estrogens. More recent studies, however, have shown that the relative risk of developing gallbladder disease among oral-contraceptive users may be minimal. The recent findings of minimal risk may be related to the use of oral-contraceptive formulations containing lower hormonal doses of estrogens and progestogens.

8. *Carbohydrate and Lipid Metabolic Effects.* Oral contraceptives have been shown to cause glucose intolerance in a significant percentage of users. Oral contraceptives containing greater than 75 micrograms of estrogens cause hyperinsulinism, while lower doses of estrogen cause less glucose intolerance. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents. However, in the nondiabetic woman, oral contraceptives appear to have no effect on fasting blood glucose. Because of these demonstrated effects, prediabetic and diabetic women should be carefully observed while taking oral contraceptives. A small proportion of women will have persistent hypertriglyceridemia while on the pill. As discussed earlier (see "Warnings" 1a. and 1d.), changes in serum triglycerides and lipoprotein levels have been reported in oral-contraceptive users.

9. *Elevated Blood Pressure.* An increase in blood pressure has been reported in women taking oral contraceptives and this increase is more likely in older oral-contraceptive users and with continued use. Data from the Royal College of General Practitioners and subsequent randomized trials have shown that the incidence of hypertension increases with increasing quantities of progestogens. Women with a history of hypertension or hypertension-related diseases, or renal disease should be encouraged to use another method of contraception. If women with hypertension elect to use oral contraceptives, they should be monitored closely, and if significant elevation of blood pressure occurs, oral contraceptives should be discontinued. For most women, elevated blood pressure will return to normal after stopping oral contraceptives, and there is no difference in the occurrence of hypertension among ever-and never-users.

10. *Headache.* The onset or exacerbation of migraine or development of headache with a new pattern that is recurrent, persistent or severe requires discontinuation of oral contraceptives and evaluation of the cause.

11. *Bleeding Irregularities.* Breakthrough bleeding and spotting are sometimes encountered in patients on oral contraceptives, especially during the first three months of use. The type and dose of progestogen may be important. Nonhormonal causes should be considered and adequate diagnostic measures taken to rule out malignancy or pregnancy in the event of breakthrough bleeding, as in the case of any abnormal vaginal bleeding. If pathology has been excluded, time or a change to another formulation may solve the problem. In the event of amenorrhea, pregnancy should be ruled out. Some women may encounter post-pill amenorrhea or oligomenorrhea, especially when such a condition was preexistent.

Precautions

1. PHYSICAL EXAMINATION AND FOLLOW UP. A complete medical history and physical examination should be taken prior to the initiation or reinstitution of oral contraceptives and at least annually during use of oral contraceptives. These physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including cervical cytology, and relevant laboratory tests. In case of undiagnosed, persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be conducted to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care. 2. LIPID DISORDERS. Women who are being treated for hyperlipidemias should be followed closely if they elect to use oral contraceptives. Some progestogens may elevate LDL levels and may render the control of hyperlipidemias more difficult. (See "Warnings" 1d.) 3. LIVER FUNCTION. If jaundice develops in any woman receiving such drugs, the medication should be discontinued. Steroid hormones may be poorly metabolized in patients with impaired liver function. 4. FLUID RETENTION. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention. 5. EMOTIONAL DISORDERS. Patients becoming significantly depressed while taking oral contraceptives should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug related. Women with a history of depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. 6. CONTACT LENSES. Contact-lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist. 7. DRUG INTERACTIONS. Reduced efficacy and increased incidence of breakthrough bleeding and menstrual irregularities have been associated with concomitant use of rifampin. A similar association, though less marked, has been suggested with barbiturates, phenylbutazone, phenytoin sodium, and possibly with griseofulvin, ampicillin and tetracyclines. 8. INTERACTIONS WITH LABORATORY TESTS. Certain endocrine-and liver-function tests and blood components may be affected by oral contraceptives: a. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. b. Increased thyroid-binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. c. Other binding proteins may be elevated in serum. d. Sex-binding globulins are increased and result in elevated levels of total circulating sex steroids and corticoids; however, free or biologically active levels remain unchanged. e. Triglycerides may be increased. f. Glucose tolerance may be decreased. g. Serum folate levels may be depressed by oral-contraceptive therapy. This may be of clinical significance if a woman becomes pregnant shortly after discontinuing oral contraceptives. 9. CARCINOGENESIS. See "Warnings" section. 10. PREGNANCY. Pregnancy Category X. See "Contraindications" and "Warnings" sections. 11. NURSING MOTHERS. Small amounts of oral-contraceptive steroids have been identified in the milk of nursing mothers and a few adverse effects on the child have been reported, including jaundice and breast enlargement. In addition, oral contraceptives given in the postpartum period may interfere with lactation by decreasing the quantity and quality of breast milk. If possible, the nursing mother should be advised not to use oral contraceptives but to use other forms of contraception until she has completely weaned her child.

Information for the Patient

See Patient Labeling.

Adverse Reactions

An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see "Warnings" section): thrombophlebitis, arterial thromboembolism, pulmonary embolism, myocardial infarction, cerebral hemorrhage, cerebral thrombosis, hypertension, gallbladder disease, hepatic adenomas or benign liver tumors.

There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: mesenteric thrombosis, retinal thrombosis.

The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug related: nausea; vomiting; gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding; spotting; change in menstrual flow; amenorrhea; temporary infertility after discontinuation of treatment; edema; melasma which may persist; breast changes: tenderness, enlargement, secretion; change in weight (increase or decrease); change in cervical erosion and secretion; diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening); intolerance to contact lenses.

The following adverse reactions have been reported in users of oral contraceptives and the association has been neither confirmed nor refuted: congenital anomalies, premenstrual syndrome, cataracts, optic neuritis, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, cerebral-vascular disease with mitral valve prolapse, lupus-like syndromes, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, impaired renal function, hemolytic uremic syndrome, Budd-Chiari syndrome, acne, changes in libido, colitis, sickle-cell disease.

Overdosage

Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Noncontraceptive Health Benefits

The following noncontraceptive health benefits related to the use of oral contraceptives are supported by epidemiological studies which largely utilized oral-contraceptive formulations containing doses exceeding 0.035 mg of ethinyl estradiol or 0.05 mg of mestranol.

Effects on menses: increased menstrual cycle regularity, decreased blood loss and decreased incidence of iron-deficiency anemia, decreased incidence of dysmenorrhea.

Effects related to inhibition of ovulation: decreased incidence of functional ovarian cysts, decreased incidence of ectopic pregnancies.

Effects from long-term use: decreased incidence of fibroadenomas and fibrocystic disease of the breast, decreased incidence of acute pelvic inflammatory disease, decreased incidence of endometrial cancer, decreased incidence of ovarian cancer.

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Term birth after midtrimester hysterotomy and selective delivery of an acardiac twin

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OBJECTIVE: Our aim was to determine whether hysterotomy and selective removal of an acardiac twin could improve the outcome of the "pump" twin.

STUDY DESIGN: A literature and case review of the outcome of the acardiac twin malformation was performed. When an acardiac malformation was diagnosed at 19 weeks' gestation the patient was monitored with weekly ultrasonographic examinations. At 23 weeks' gestation, no blood flow could be demonstrated to the acardiac twin and it was thought that the continued presence of the acardiac twin posed a risk to the "pump" twin. A midtrimester hysterotomy was performed and the acardiac twin was delivered.

RESULTS: After the midtrimester hysterotomy, the pregnancy progressed to term and a healthy female infant was delivered by elective cesarean section at 37 weeks' gestation.

CONCLUSION: Midtrimester hysterotomy may be a useful intervention in cases of twinning when one fetus is a threat to the health of the other. (AM J OBSTET GYNECOL 1992;167:33-7.)

Key words: Acardiac twin, fetal surgery, midtrimester hysterotomy, term delivery, acardiac in triplets

The acardiac twin malformation has been reported to occur in 1 in 34,600 pregnancies and is unique to multiple gestations containing monozygotic twin pairs.[1] The epidemiologic features of the acardiac deformity have been studied by James,[2] and his review of 340 cases revealed that the complication occurs considerably more frequently among monoamniotic than diamniotic monochorionic twins. Acardiac twins occur in 1% of monozygotic twins but three times more frequently in monozygotic twin pairs in higher-order multiple births. A slight female predominance has also been noted.[1-5]

Four morphologic types have been described by Napolitani and Schreiber[3]: (1) The condition is designated hemiacardius when the fetus has some rudimentary cranial structures. (2) Holoacardius acephalus is the most commonly described. These fetuses lack cranial and thoracic organs. Pelvic bones and lower extremities are well developed and upper extremities may or may not develop. (3) Holoacardius acormus is the rarest form and consists of a head without a body. (4) Holoacardius amorphus is the least-developed type with no recognizable human form.

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The acardiac malformations are uniformly fatal in the affected twin, and mortality in the co-twin has been reported by Moore et al.[4] to be as high as 55%. Moore et al. tabulated the perinatal outcomes of 49 pregnancies complicated by the presence of acardiac twinning. The principal problems were congestive heart failure of the "pump" twin (nonacardiac twin), polyhydramnios, and preterm labor. In that series therapeutic amniocentesis was the only invasive form of management attempted. Their findings of 50% mortality provide a standard against which newer management schemes can be compared. Furthermore, only 25% of their pregnancies progressed to term.[4]

Antenatal diagnosis of the condition by ultrasonography was first reported in 1978, allowing the first opportunity to consider antepartum management of this condition.[5]

The described therapeutic management in cases of one acardiac twin diagnosed antenatally includes the following: (1) continuation of the pregnancy in the interest of the normal twin, (2) termination of the entire pregnancy in spite of the presence of one normal fetus, (3) selective delivery of the abnormal fetus,[6] (4) serial amniocentesis for treatment of polyhydramnios in the "pump twin,[7] (5) maternal administration of digoxin in an effort to treat cardiac failure in the "pump" twin,[8] and (6) placement of a thrombogenic coil to obstruct blood flow to the acardiac fetus. The use of the thrombogenic coil has been reported by Porreco et al.[9] in a case of occlusion of the umbilical artery of the acardiac

twin. The "pump" twin was delivered at 39 weeks and was healthy. Personal communication with a physician from Tokyo, Japan, on the use of the thrombogenic coil has shown somewhat disappointing results. He has treated four patients with this method and found it to be effective in only 50% of patients. In addition, he and others (personal communications) have found the obstruction created by the coil to be transient in some cases.

Umbilical cord clamping via an intrauterine laparoscopic procedure has been proposed and was planned for a patient who was delivered prematurely before the procedure could be performed.[8]

Successful selective delivery of the acardiac twin via hysterotomy and continuation of the pregnancy with the viable fetus has been reported by Robie et al.[6] That pregnancy was delivered prematurely at 32 weeks. Our report describes hysterotomy and selective delivery of an acardiac twin, after which the pregnancy progressed to term before elective delivery was performed. The only other term delivery after a midtrimester hysterotomy was performed by Beck et al.[10] when one twin was removed because it was affected with Down syndrome.

First, we describe a case of a triplet pregnancy with an acardiac fetus, managed conservatively, in which the pump triplet died neonatally in spite of a near-term delivery.

Case reports

Case 1. A 20-year-old primigravid woman was referred to our center at 28 weeks of gestation. The fundal height at that time measured 43 cm, and ultrasonographic evaluation revealed a triplet gestation. Triplet A was in a cephalic presentation and was shown to be appropriate for gestational age at 28 weeks. Polyhydramnios was present. Triplet B was concordant with A and in a cephalic presentation. Amniotic fluid volume was normal. Triplet C was in a transverse lie in the fundus. The femur length of triplet C corresponded to 25 weeks' gestation, and no thorax, upper extremities, or head was present. This fetus shared a circulation with triplet A. Preterm labor began at 28 weeks, and successful tocolysis was achieved with magnesium sulfate. Later a switch was made to an oral β-adrenergic receptor agonist. At 34 weeks the patient presented with spontaneous rupture of membranes and in active labor; delivery was accomplished. Triplet A, the pump triplet, weighed 1630 gm and had Apgar scores of 8 and 9 at 1 and 5 minutes, respectively. This triplet did well for the first 24 hours and then went into intractable heart failure and died. Autopsy of that neonate failed to reveal any structural cardiac abnormality other than massive cardiac dilatation as a secondary effect of heart failure. No other developmental anomalies were present. Triplet B had an uneventful neonatal course.

Case 2. A 30-year woman, gravida 4, para 3-0-0-3, presented for her first prenatal visit at 14 weeks' gestation. The patient became extremely orthopneic and dyspneic at 19 weeks and was uncomfortable presumably because of polyhydramnios. Ultrasonographic examination revealed a monochorionic, diamniotic twin gestation with an acardiac fetus approximately two thirds the size of the pump twin. The pregnancy was diamniotic, and polyhydramnios was seen in the sac of the pump twin only. The umbilical cord of the acardiac twin could be seen originating from a portion of the pump twin's cord. Doppler flow studies demonstrated reversed flow in the acardiac twin's umbilical cord. Alternatives of management presented to the patient included (1) termination of the entire pregnancy, (2) expectant management, and (3) selective removal of the malformed fetus. The presence of an anterior placenta that covered the entire anterior surface of the uterus prevented the possibility of any percutaneous approach. Although the thrombogenic coil has been mentioned here, this case occurred before the report of the Porreco et al. appeared in the Lancet, and therefore that option was not offered to the patient.

Both the institutional ethics committee and the institutional review board were convened, and they determined that if the pump twin became at significant risk selective removal of the acardiac twin would be appropriate. Amniocentesis was performed at the nineteenth week of gestation for chromosomal evaluation and relief of the symptomatic polyhydramnios. Fluid rapidly reaccumulated after therapeutic amniocentesis at 19 weeks. It was our opinion that further therapeutic amniocentesis was not likely to be effective, in contrast to cases of twins with the twin-twin transfusion syndrome, because the hemodynamic situations are vastly different. No further invasive procedures were attempted to preclude iatrogenic premature rupture of membranes.

Karyotypic evaluation revealed that both twins had identical karyotypes with inversion of chromosome 10:46,XX,inv(10)(p12q25). Further studies revealed that the mother and her two tested children carry the same inversion and were phenotypically normal.

Weekly ultrasonographic examinations showed that, inspite of therapeutic amniocentesis, the polyhydramnios progressively worsened. Evaluation at 23 weeks' gestation demonstrated skin edema and total cessation of movements, as well as diminished blood flow in the cord of the acardiac twin. Two days later skin edema drastically worsened in the acardiac fetus, and flow through the umbilical cord could no longer be consistently demonstrated. There was concern regarding a dead twin in a monochorionic twin pregnancy. The institutional ethics committee and institutional review board were convened, and they determined that, because of the risk to the pump twin, selective delivery of the acardiac fetus would be appropriate.

The team was assembled in preparation for a hysterotomy the following morning. Pretreatment with indomethacin the night before was initiated. Enflurane was used to provide deep general anesthesia and furnish adequate uterine relaxation. Through a large mid-

line incision, the uterus was exteriorized to allow an approach to the posterior surface of the uterus. Sterile intraoperative ultrasonography was used both to determine the location for the uterine incision and to monitor the pump twin during the procedure. After the uterus was entered, the amniotic membrane was sutured to the incision line, and the acardiac fetus was then extracted. The umbilical cord of the abnormal twin was identified and tied off with Merseilene tape. The then-empty sac of the acardiac twin was instilled with warmed saline solution mixed with nafcillin. The uterus was then closed in layers after the application of fibrin glue to provide a watertight seal and control of hemostasis. Postmortem evaluation of the acardiac fetus revealed substantial edema in a female hemiacardius. The umbilical cord demonstrated some thrombosis.

Postoperative care included the continuation of indomethacin and magnesium sulfate until conversion to an oral β-adrenergic receptor agonist could be accomplished. Uterine activity was minimal during the patient's entire postoperative course. The patient was discharged on the seventh postoperative day on a regimen of terbutaline and home uterine monitoring.

The patient was examined weekly, and the polyhydramnios resolved along with the orthopnea. The remaining fetus continued to develop normally, and the remainder of the patient's prenatal course was completely uneventful. At 38 weeks gestation, after an amniocentesis was performed to assure pulmonary maturity, the fetus was electively delivered by cesarean section. Examination of the posterior surface of the uterus, at the site of the prior hysterotomy, showed a dense adhesion to a portion of small bowel. The myometrium was otherwise well healed and normal in appearance. A female infant weighing 3040 gm was delivered. The Apgar scores were 9 and 9 at 1 and 5 minutes, respectively. The placenta was expressed out without difficulty, and the vascular anastomosis with the acardiac twin can be seen in Fig. 1. Physical examination of the infant was unremarkable except for a very small membranous ventricular-septal defect that has closed spontaneously. The mother had an uneventful postoperative recovery. The child is now 8 months old and is developing normally without any medical problems.

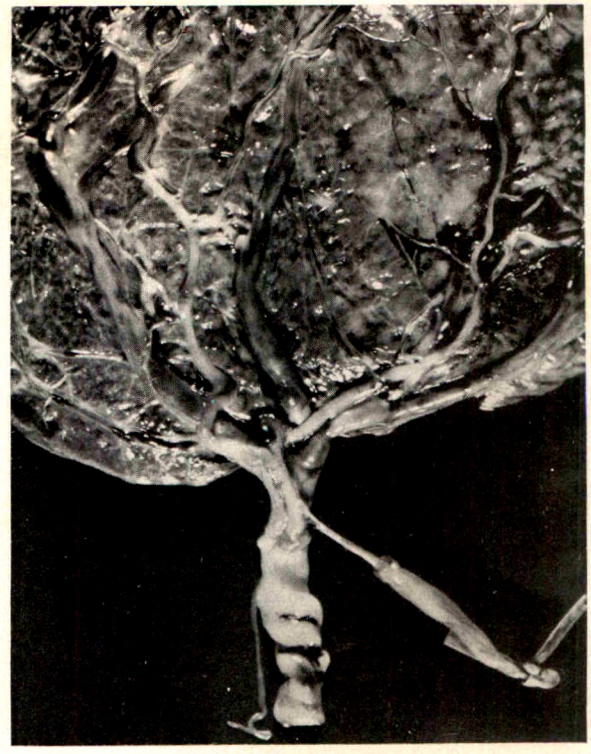


Fig. 1. Term placenta with cord insertion of acardiac twin.

Comment

This case represents a term delivery after hysterotomy as part of fetal therapy. The contemplation of open fetal surgery poses a dilemma from the physician's perspective with respect to the best way to balance the needs of the fetus and their implications for the mother. The use of open fetal surgery automatically commits the mother to two major operations, a hysterotomy followed by a cesarean section, with all of their potential associated morbidity. For these reasons case selection is especially important.

The disease entity in which surgery is selected should have a high likelihood of resulting in a functionally normal child and should have an otherwise dismal prognosis if treated by conventional noninvasive measures. Toward this end, the fetus should be completely evaluated by both a chromosome analysis, because there are reported cases of chromosomal anomalies in the pump twin,[11] and a detailed ultrasonographic study. Similarly, the mother must be prepared both physically and emotionally to cope with the restrictive postoperative prenatal care program that is essential to a successful outcome.

The selection of acardiac twins as a model for open fetal surgery is appropriate for several reasons. Acardiac twins, although a rare occurrence, have a large body of literature written about their natural outcome.[1-4] Removal of the acardiac twin does not create an ethical dilemma because it cannot survive outside the uterus. The pump twin is generally normal and can be expected to function normally if it can be delivered as a mature fetus in good condition. Furthermore, parameters have been developed to predict the likely consequences of nonintervention[4] and provide a data base from which conclusions on outcome can be drawn. In the series of Moore et al.[4] no invasive therapeutic modalities were used other than amniocentesis to relieve polyhydramnios, which was performed in four of 49 cases. Those authors described the relationship between the weight of the acardiac twin and that of the

pump twin to predict the outcome. When the weight of the acardiac twin exceeded 70% of that of the pump twin, congestive heart failure developed in 100% of the pump twins. When the acardiac/pump twin ratio was ≥50%, 64% of the pump twins died. Hydramnios was strongly associated with preterm delivery and congestive heart failure. Eighty-two percent of patients with hydramnios experienced preterm labor, and congestive heart failure developed in 78%. When the acardiac/pump twin ratio was ≤25%, the likelihood of adverse outcome was very small.

Deciding on the appropriate time to intervene in this case was based on several assumptions. It was our opinion that the pump twin was likely to fare better if surgery was performed before the fetus became hydropic. The experience of Platt et al.[8] suggests that surgery should be performed before the onset of premature labor, because once premature labor begins, it may not be possible to stop it. Anderson et al.[12] and others have reported that death of one monozygotic twin may lead to disruptive structural defects in the central nervous system, gastrointestinal tract, renal system, and terminal limbs, as well as death, in its co-twin. The incidence of these complications has been reported as 46%.[13]

A longitudinal midline skin incision was used, rather than a transverse incision, which is generally used by Harrison (and his group[14] in San Francisco) to allow for easier exposure to the posterior surface of the uterus. The skin incision should not be allowed to compromise the procedure for cosmetic reasons.

Care was taken to plan the uterine incision as far away from the placental edge as possible and yet make it large enough to remove the acardiac twin without entering the sac of the pump twin.

Monitoring the pump twin during the surgery provides a unique problem since it is not readily accessible. This was done by continuous ultrasonographic visualization and with intermittent Doppler readings to measure the systolic/diastolic ratio. Monitoring of the pump twin was done because there was concern that exteriorization of the uterus might compromise uteroplacental blood flow or potentially compress the fetus against the umbilical cord.

Reversed umbilical artery blood flow to the acardiac twin has been described by Pretorius et al.,[15] Kirkinen et al.,[16] and Benson et al.[17] Different investigators have arrived at different conclusions in their study of umbilical artery waveforms demonstrated in the twin arterial perfusion sequence. Farmakides et al.[18] and Sherer et al.[19] obtained discordant umbilical waveforms, while Giles et al.[20] determined that twin transfusion syndrome with discordant growth is associated with the presence of concordant waveforms. Cyclic alterations of umbilical artery waveforms in a monochorionic gestation were described by Erskine et al.[21] Pretorius et al.[22] noted no consistent patterns of the umbilical waveforms in eight cases of twin arterial perfusion sequence. Similarly, Neilson et al.[23] were unable to discern any consistent pattern of umbilical artery waveforms resulting from vascular anastomoses in monochorionic gestations. In our case both reversed flow and discordant umbilical artery waveform patterns were present.

The chorioamniotic membranes were handled in a different way than described by Robie et al.[6] The membranes were sutured to the hysterotomy incision line rather than being stapled. We chose to suture them to avoid being confined by the size of the staples. Chromic suture material rather than polyglycolic was used.

Great care was taken to assure that no instruments placed on the uterine incision to control hemostasis ever incorporated any portion of the membranes.

Fibrin glue was used to seal the chorioamniotic membranes against leakage. This substance was first used for this indication by Genz[24] in 1979, when he treated premature rupture of membranes at 16 and 23 weeks. Electron microscopy suggested that this substance promotes the regeneration of amniotic membrane at the site of the defect. Adamyan et al.[25] in their review of fibrin glue in obstetrics and gynecology have shown it to promote reparative regeneration and to decrease adhesion formation. We were impressed by the excellent hemostasis that could be achieved at the hysterotomy site.

One can only speculate what factors allowed this case to go to term, whereas most other cases of open fetal surgery have ended in premature labor. Several factors may be of significance, such as refilling the sac of the acardiac twin to let the uterus gradually decrease in size rather than abruptly. An additional advantage to refilling the acardiac twin's sac was to provide a liquid buffer at the hysterotomy site against the movements of the pump twin. Timing the surgery to occur before the pump twin was significantly compromised likely improved its ability to tolerate the stress of surgery. Having an established team specifically dedicated to performing this procedure facilitated a smooth flow from start to finish. Finally, having a highly motivated and compliant patient certainly contributed to our success.

Aggressive surgical management can result in successful intervention in pregnancies where ultrasonographic monitoring indicates that a fetus is in imminent danger of significant morbidity or mortality as elucidated by Moore et al.[4] When the acardiac/pump twin ratio is ≤25%, conservative management generally will result in a term delivery without development of hydrops. The first case presented illustrates that near-term delivery is not the only criterion for a good outcome. The fetus must also be hemodynamically able to

withstand the change in circulation to an extrauterine environment. In those circumstances where surgery can correct the anatomic defect that cannot wait for delivery, patients should be referred to centers with experience in these techniques. Centralized referral of these rare cases is likely to improve the outcome and facilitate meaningful data collection on the safety of this kind of alternative.

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Idiopathic myelofibrosis in pregnancy: A case report and review of the literature

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Idiopathic myelofibrosis is a rare myeloproliferative disorder characterized by excessive accumulation of connective tissue in the bone marrow n association with anemia, splenomegaly, and extramedullary hematopoiesis. The cause of this disease is unknown, and the prognosis is generally poor. To our knowledge, this is the first case report of a patient with idiopathic myelofibrosis who carried a term pregnancy. In spite of the increased perinatal risks, a favorable outcome was possible with close antepartum surveillance. (AM J OBSTET GYNECOL 1992;167:38-9.)

Key words: Idiopathic myelofibrosis

Myelofibrosis is a myeloproliferative disorder characterized by an abnormal accumulation of connective tissue in the bone marrow, extramedullary hematopoiesis, and leukoerythroblastic anemia. The cause of this disease is unknown,[1] and in the absence of an underlying malignant process the term idiopathic myelofibrosis is used.[2]

The signs and symptoms are often vague; therefore definitive diagnosis requires a bone marrow biopsy. The prognosis for these patients is extremely poor, with a 5-year survival of 9 to 30 months, often complicated by infections, cardiovascular diseases, and thrombohemorrhagic events.[2] The physiologic alterations of pregnancy may further complicate the clinical course of patients with idiopathic myelofibrosis. These patients are therefore at increased risk for poor perinatal outcome.

To our knowledge, our report describes the first patient with primary myelofibrosis who was delivered of a term infant and includes our recommendations for a multidisciplinary approach and close antepartum surveillance of these pregnancies.

Case report

A 23-year-old primiparous woman was referred to University Hospital for prenatal care at 15 weeks' gestation. Her medical history was significant for idiopathic myelofibrosis with diffuse osteosclerosis diagnosed 3 years before this pregnancy. Her history was also significant for a leukoagglutinin reaction to a packed red blood cell transfusion in 1988. The family history was significant for myelofibrosis in her brother. Management by hematologists had been supportive before this pregnancy, with the use of hematinics and analgesics for joint pain.

On physical examination she was noted to have mild splenomegaly. Ultrasonography revealed a single appropriate-for-gestational-age fetus. Laboratory workup showed white blood cell count 5600/mm³, hemoglobin 6.4 gm/dl, hematocrit 21.8%, and platelet count 88,000 μm/L. Anemia workup revealed a normochromic, normocytic pattern. The reticulocyte count was in the normal range, but an elevated serum erythropoietin level of 2140 mU/ml (normal <19 mU/ml) was observed.

The patient's constitutional symptoms (joint pain) did not worsen during pregnancy. The lowest hematologic parameters were at 28 weeks' gestation with hemoglobin 6.3 gm/dl, hematocrit 19.2%, and platelet count 88,000 μm/L. Fetal growth was adequate as documented by serial ultrasonography, and all studies of fetal well-being were normal. After fetal lung maturity was documented, elective induction of labor was performed at 36 weeks, followed by spontaneous vaginal delivery of a 3100 gm female infant, with Apgar scores of 8 and 9 at 1 and 5 minutes, respectively. The neonate's nursery course was uncomplicated. The patient is currently undergoing management of her case by her hematologist 1 year after delivery without worsening disease.

Comment

Myelofibrosis was first described in 1879 by Hueck. Theories proposed to explain the cause of idiopathic myelofibrosis suggest that the disease occurs as a result of the bone marrow's reaction to neoplastic invasion, viruses, toxins, ionizing radiation, or chemotherapeutic agents.[1, 2] Although there is no known pattern of in-

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heritance for the disease, karyotypic abnormalities among affected individuals suggest a genetic basis.[1] In our case, the patient's brother was diagnosed with the same disease 2 years earlier.

Although many patients with idiopathic myelofibrosis may be asymptomatic at the time of diagnosis, some are seen with fatigue, weight loss, dyspnea, and fever.[2] The most common medical complications noted in idiopathic myelofibrosis patients are infections (63%), cardiovascular disease (59%), and thrombohemorrhagic complications (49%).[2]

Therapy for asymptomatic individuals is generally supportive. Bone marrow transplantation, splenectomy, androgens, and chemotherapy have been used to treat some individuals with limited success.[1] Recently, α-interferon and erythropoietin have been recommended by some investigators. The elevated level of erythropoietin in our patient eliminated its potential use as an alternative treatment. The prophylactic use of blood transfusions has been recommended for some patients with chronic anemia; however, this does not generally improve perinatal outcome.

In summary, patients with idiopathic myelofibrosis should be carefully monitored during pregnancy for maternal and fetal well-being. Our experience showed that a favorable outcome is possible with close antepartum surveillance. However, the long-term prognosis of an intercurrent pregnancy on this disease is uncertain.

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Oral contraceptive use and the incidence of cervical intraepithelial neoplasia

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OBJECTIVE: Our objective was to examine the relationship between oral contraceptive use and the incidence of cervical intraepithelial neoplasia.
STUDY DESIGN: In a prospective follow-up study of 6622 women participating in the Second Tromsö Study conducted in 1979 and 1980 in Tromsö, Norway, women aged 20 to 49 years answered a questionnaire regarding their smoking history, dietary habits, alcohol consumption, and oral contraceptive use. They were then followed for 10 years with data from the Pathology Registry of the University Hospital.
RESULTS: The age-adjusted incidence rate of cervical intraepithelial neoplasia was 897 per 100,000 person years among noncurrent and 1295 per 100,000 person years among current oral contraceptive users as of 1979. After adjusting for age, marital status, smoking, and frequency of alcohol intoxication the relative rate for current users was 1.5 (95% confidence interval 1.1 to 2.1), and the relative rate for past users was 1.4 (95% confidence interval 1.0 to 1.8), as compared with those who had never used oral contraceptives before 1979.
CONCLUSION: These findings support the hypothesis that the occurrence of cervical intraepithelial neoplasia is increased by oral contraceptive use. (Am J Obstet Gynecol 1992;167:40-4.)

Key words: Oral contraceptives, cervical dysplasia, follow-up studies, Norway, cervical neoplasms

In two extensive reviews it was concluded that a weak positive association seems to be emerging between oral contraceptive use and the risk of cervical neoplasia but that this association may be due to bias and confounding.[1,2] The relationship remains controversial because recent epidemiologic studies continue to yield conflicting results.[3-7] It has been proposed that the findings of positive studies reflect enhanced detection of cervical intraepithelial neoplasia among oral contraceptive users rather than a causal association.[8]

We observed that among women who participated in the Second Tromsö Study and who were current oral contraceptive users as of 1979, grade 3 cervical intraepithelial neoplasia incidence during the following 10 years was 1.4 times higher than among nonusers.[9] Although this increased incidence among oral contraceptive users was not explained by confounding factors such as cigarette smoking or the number of cytologic examinations (Papanicolaou smears), it lacked statistical significance. In this report we expand the previous analysis by evaluating the relationship between oral contraceptive use and all cervical intraepithelial neoplasia grades and by adding information from the Third Tromsö Study.

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Material and methods

Between 1979 and 1980 all women (n = 9906) aged 20 through 49 years and all men (n = 11,423) aged 20 through 54 years living in the municipality of Tromsö were invited to participate in the Second Tromsö Study. Complete details of the study methods are given elsewhere.[10] The participants filled out one questionnaire at the screening facility and another at home. The first questionnaire concerned disease history and aspects of living habits, including cigarette smoking and oral contraceptive use. The second questionnaire elicited information on dietary habits, alcohol and coffee consumption, previous diseases, and social and psychologic conditions. The participants were instructed to return this questionnaire by mail. The Third Tromsö Study was conducted in 1986 and 1987. At this survey the questionnaires were modified to add information on use and duration of oral contraceptives and intrauterine contraceptive devices, age at first marriage or cohabitation, and age at first pregnancy.[11]

Women participating in the Second Tromsö Study (n = 8134) with no history of the disease were followed for the development of cervical intraepithelial neoplasia or cervical cancer. The follow-up was made possible by linkage of their national personal identification

numbers with the computerized information in the Pathology Registry of the University Hospital in Tromsö. This registry provides complete records of all cytologic and histologic diagnoses made in the county where Tromsö is located. Altogether 7838 (96%) women from the Second Tromsö Study had a cervical specimen recorded in the registry during 1980 through 1989.

Criteria for inclusion in the analytic cohort were (1) no diagnosis of cervical intraepithelial neoplasia or cancer of the cervix before Jan. 1, 1980, and (2) at least one normal cervical specimen within 3 years before enrollment in the Second Tromsö Study or after enrollment. Excluded from follow-up were 528 women who had a diagnosis of cervical intraepithelial neoplasia or invasive cancer of the cervix before enrollment or as their first cervical specimen recorded in the registry. Follow-up began on Dec. 31, 1979, for 5415 women who had a normal specimen during the previous 3 years and on the date of the first normal specimen recorded for the remaining 1895 women. However, 688 women had no subsequent specimens and contributed no information to the follow-up study. Thus the analysis is restricted to 6622 women (81% of all participants in the Second Tromsö Study). Follow-up ended on the date of diagnosis of cervical intraepithelial neoplasia, autopsy or hysterectomy, or their last cervical specimen, whichever was earliest.

Incident cervical intraepithelial neoplasia cases were classified according to the first diagnosis. Thus, if during the study period a woman had a first diagnosis of grade 1 disease that later progressed to grade 3, she was counted only once as a grade 1 case.

Person years of follow-up were assigned to categories of potential determinants of risk for cervical intraepithelial neoplasia. Incidence rates were computed by dividing the number of cases by the number of person years in that category. Age-adjusted rates were calculated by the direct method, using the age distribution of person-years in the entire analytic cohort as the standard.[12] Data analysis included an evaluation of incidence rates by age, marital status, education, age at first pregnancy, age at first marriage or cohabitation, frequency of fruit and vegetable consumption, frequency of fish consumption, frequency of drunkenness, cigarette smoking, number of specimens, and time between specimens. Cases and person years were classified into current and noncurrent oral contraceptive users as of 1979 on the basis of information obtained from the Second Tromsö Study. More detailed information on the history of oral contraceptive use was available for 4912 (74%) who also participated in the Third Tromsö Study. Data from the third study were used to ascertain whether women who were nonusers at the time of enrollment into the second study had ever used oral contraceptives before that date (past users). Thus cases and person years were reclassified into never users, past users, and current users. Women from the Second Tromsö Study who were noncurrent users at enrollment and who did not participate in the third study were classified as "other noncurrent users." It is likely that this group is a mixture of never and past users.

The relative rate was used to compare category-specific incidence rates. Relative rates were also estimated with the Cox proportional hazards regression model to adjust simultaneously for the effects of several potential confounders.[13] The follow-up experience of subjects was analyzed by blocking on the number of specimens (one or two, three or four, five or six, seven or more) that they had accumulated during the follow-up period. This blocking was done because the likelihood that a diagnosis of cervical intraepithelial neoplasia is made during the observation period increases with more frequent screening. However, the results did not change materially whether the analysis was performed without the blocking factor or by including the number of specimens as covariates.

Poisson regression models were also used to obtain relative rate estimates adjusted for time between screens (and the confounding variables included in the proportional hazards model) and to evaluate interaction among potential risk factors. The significance of a trend in the incidence of cervical intraepithelial neoplasia with increasing levels of a factor was evaluated by assigning equally spaced ordinal scores to categories of the factor and including the score as a continuous variable in a Poisson regression model.[14] Multiplicative terms between oral contraceptive use and possible confounders were included in the model to evaluate interaction.

Results were considered statistically significant if the p value was ≤ 0.05. The 95% confidence intervals are reported throughout the paper. The proportional hazards regression analyses were performed with the PHGLM procedure of the SAS statistical package.[15] The Poisson regression analyses were performed with the EGRET statistical package.[16]

Results

During the 43,316 person years of observation, 401 incident cases (354 women with grade 1 or 2 cervical intraepithelial neoplasia as their first abnormal diagnosis, 44 with grade 3 cervical intraepithelial neoplasia, and three with cervical cancer) were identified. These women are included in all analyses. We note that inclusion of only the 354 women with grade 1 or 2 cervical intraepithelial neoplasia in the analysis did not change the results materially.

Seventy percent of the women were married, their median years of schooling were 11 (7 to 23), and at the beginning of the follow-up their median age was 31 (20

Table I. Distribution of study subjects according to selected characteristics and prevalence of current oral contraceptive use in a cohort of 6622 women, Tromsö, Norway, 1980 through 1989

Characteristics	*Cohort (n = 6622)*	*Prevalence of current* oral contraceptive use (%)*
Age (yr)		
20-24	1132	21
25-29	1451	11
30-34	1474	7
35-39	1124	5
40-44	789	3
45-49	652	1
Marital status		
Married	4578	6
Divorced or widowed	426	9
Single	1581	17
Smoking status		
Never	2247	7
Past	1291	8
Current	3084	11
Intoxication by alcohol		
Never	2879	6
Less than monthly	2463	12
Monthly or more	452	14

*As of 1979.

Table II. Multivariate* relative rate estimates of cervical intraepithelial neoplasia with 95% confidence interval, according to selected characteristics, in a cohort of 6622 women, Tromsö, Norway, 1980 through 1989

Characteristic	*Relative rate*	*95% Confidence interval*
Marital status		
Married	1.0	
Divorced or widowed	1.4	0.9-2.1
Single	1.6	1.2-2.1
Smoking status		
Never	1.0	
Past	1.0	0.7-1.4
Current	1.6	1.2-2.1
Frequency of intoxication by alcohol		
Never	1.0	
Less than monthly	1.4	1.1-1.8
Monthly or more	1.9	1.3-2.7

*Based on 348 cases from Cox proportional hazards model with age group, marital status, smoking status, frequency of intoxication by alcohol, and oral contraceptive use blocking for number of specimens.

to 64) years. Only 9% of the women were current oral contraceptive users in 1979 and 1980.

For never, past, and current oral contraceptive users the mean follow-up time was 7 years and the average number of specimens obtained was five. The women who were noncurrent users in 1979 with missing information on ever use (women who did not participate in the Third Tromsö Study in 1986 and 1987) had an average number of four specimens obtained during the mean follow-up period of 5 years.

Prevalence of oral contraceptive use was higher among women of young age, among unmarried women, among cigarette smokers, and among women reporting frequent intoxication by alcohol (Table I). The relative rate estimates of cervical intraepithelial neoplasia from the proportional hazards regression models show that these women also have a significantly increased risk for cervical intraepithelial neoplasia (Table II). Thus each of these factors was considered a potential confounder of the oral contraceptive–cervical intraepithelial neoplasia association, and adjustment was made for each in a multivariate proportional hazards model. No meaningful associations were found between cervical intraepithelial neoplasia and years of schooling, fruit and vegetable consumption, or fish consumption.

The age-adjusted incidence rate of cervical intraepithelial neoplasia was 897 per 100,000 person years among noncurrent and 1295 per 100,000 person years among current oral contraceptive users as of 1979 ($p = 0.05$). A multivariate proportional hazards regression model on 348 cases with complete information on potential confounders (age in 5-year groups, marital status [married, divorced-widowed, single], smoking status [never, past, current], frequency of intoxication by alcohol [never, less than monthly, monthly or more]) yielded increased relative rate estimates of cervical intraepithelial neoplasia among noncurrent, past, and current oral contraceptive users as of 1979 as compared with never users. Women starting at an earlier age were at an increased risk as compared with those starting later. An ordinal trend test across the four categories for age started oral contraceptive use yielded a p value of 0.05 (Table III).

Women who married or cohabited for the first time at a young age had an increased risk for cervical intraepithelial neoplasia. This association was, however, explained by marital status. Neither did ever using intrauterine contraceptive devices or age started intrauterine contraceptive device use explain the oral contraceptive–cervical intraepithelial neoplasia association reported.

None of the two-way interaction terms between oral contraceptive use and age, marital status, smoking, drunkenness, time between screens, and number of screens was statistically significant or meaningfully affected the relative rates presented above.

Comment

The results of this follow-up study suggest that both current and past oral contraceptive users experience a

Table III. Multivariate relative rate estimates of cervical intraepithelial neoplasia according to various measures of oral contraceptive use in a cohort of 6622 women, Tromsö, Norway, 1980 through 1989

Oral contraceptive use	*Relative risk**	*95% Confidence interval*
Never	1.0	
No information†	1.3	1.0-1.8
Past	1.4	1.0-1.8
Current	1.5	1.1-2.1
Age started, ever users‡		
>24 yr	1.1	0.7-1.8
20-24 yr	1.5	1.1-2.0
<20 yr	1.3	0.9-1.9

*Based on 348 cases from Cox proportional hazards model with age group, marital status, smoking status, frequency of intoxication by alcohol, and oral contraceptive use blocking for number of specimens.

†These women were noncurrent users in 1979, missing information on ever use.

‡Based on 242 cases from Cox proportional hazards model with complete covariate information. Trend test with four levels (never, three categories with age started oral contraceptive use), $p = 0.05$.

higher incidence of cervical intraepithelial neoplasia than do those who never used oral contraceptives. This finding is similar to those of four[17-20] of five previous follow-up studies[17-21] that evaluated the oral contraceptive–cervical intraepithelial neoplasia hypothesis.

The present study also suggests a relationship between age at start of oral contraceptive use and cervical intraepithelial neoplasia incidence. This association may in fact reflect an increasing trend of cervical intraepithelial neoplasia incidence with duration of oral contraceptive use. Three of the previously mentioned follow-up studies found a trend of increasing incidence with duration of oral contraceptive use.[17-19]

A major strength of this study is that it originates from a population-based survey with a high attendance rate. Thus the women constituting the cohort should be fairly representative of all women of similar age in the region. Strengths related to the prospective follow-up design are that women were known to be free of cervical intraepithelial neoplasia at enrollment and information on potential risk factors was collected before diagnosis. The oral contraceptive use among subjects was ascertained before outcome and hence is not subject to differential anamnestic bias, which may affect the results of case-control studies. Another strength of this study is the ability to control for confounding variables such as marital status, smoking status, and freqency of drunkenness. Because the number of Papanicolaou smears and the time between smears were also controlled for, it is unlikely that our results are explained by detection bias.

On the other hand, misclassification may result from higher rates of false-positive tests (from higher incidence of vaginal infections and cervical erosions) in oral contraceptive users. This misclassification would result in a spuriously high incidence of low-grade lesions (grade 1). We found that the incidence of grade 3 also was increased among oral contraceptive users.[9] Also, the excess cervical intraepithelial neoplasia incidence should be experienced only by current users, whereas incidence is increased among past oral contraceptive users.

The most important limitation of this study is the lack of information on sexual behavior. As in the other follow-up studies, it was not feasible to collect such data. However, it is likely that this source of confounding was partially controlled for in the analysis by adjusting for marital status and frequency of drunkenness. Information on sexual behavior is easier to collect in case-control studies. Two recent case-control studies found a positive association between oral contraceptive use and cervical intraepithelial neoplasia after controlling for number of sexual partners.[6-7]

In conclusion, the current study supports the hypothesis that the occurrence of cervical intraepithelial neoplasia is increased by oral contraceptive use.

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Calcium homeostasis in pregnant women receiving long-term magnesium sulfate therapy for preterm labor

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OBJECTIVES: The hypothesis of this study is that calcium homeostasis and bone mineralization are altered in pregnant women receiving long-term therapy with magnesium sulfate as compared with similar women not receiving magnesium sulfate to control preterm labor.
STUDY DESIGN: Thirty-nine women between 24 and 32 weeks' gestation, matched for age, race, and duration of bed rest, were enrolled. Indices of calcium homeostasis in serum and urine were measured serially, and bone mineralization of the distal radius was measured at 1 and 11 weeks post partum.
RESULTS: Magnesium therapy was administered for a mean duration of 26 ± 14 days and a cumulative dose of 1405 ± 963 gm. Serum concentrations of magnesium, phosphorus, and parathyroid hormone increased and those of calcium decreased from baseline values in the magnesium sulfate group and remained uniform throughout the 3-week investigation. The serum magnesium, phosphorus, parathyroid hormone, and calcium concentrations in the control group were unchanged during the study and differed significantly from those in the magnesium sulfate group ($p < 0.001$). Urinary output of magnesium, calcium, and copper was significantly greater in the magnesium sulfate group than in the control group throughout the study. Urinary losses of calcium in the magnesium sulfate group, approximately 800 to 900 mg/day, were substantial. Although radius bone density 1 week post partum did not differ between groups, the change in bone density from 1 to 11 weeks post partum was significantly lower in the magnesium sulfate group than in controls.
CONCLUSIONS: These data suggest that calcium homeostasis is altered during and after long-term magnesium sulfate therapy. The marked, prolonged urinary calcium losses may affect maternal bone mineralization. (Am J Obstet Gynecol 1992;167:45-51.)

Key words: Magnesium sulfate, calcium metabolism, bone mineralization

Tocolytic therapy with magnesium sulfate was originally proposed for 24 to 72 hours to delay preterm delivery until either the maximal effect of maternal glucocorticoid treatment on fetal lung maturation was achieved or β-sympathomimetic therapy was substituted.[1] This short-term $MgSO_4$ therapy is known to perturb calcium homeostasis, causing such imbalances as hypocalcemia and hypercalciuria.[2-5] These effects, reported in women with either pregnancy-induced hypertension or preterm labor, are reversible when $MgSO_4$ therapy is discontinued.[3, 4]

Long-term $MgSO_4$ tocolytic therapy recently has been described as safe and effective.[6] Tertiary care centers have adopted protocols for administering long-term $MgSO_4$ to delay preterm birth.[7] Scant published information exists regarding the effects of long-term $MgSO_4$ therapy on maternal calcium homeostasis. Two case reports raised concern about the safety of this therapy.[8, 9] Lamm et al.[8] reported on two newborn infants with metaphyseal lesions after 9 and 13 weeks, respectively, of maternal antenatal $MgSO_4$ therapy. Both infants manifested hypocalcemia and were considered by the authors to have congenital rickets. Cumming and Thomas[9] reported on an infant who had a discrete band of osteopenic metaphyseal bone. The infant, born to a mother receiving $MgSO_4$ for 13 weeks before delivery, had a normal serum calcium concentration and did not have rickets. The observations are consistent with experiments in vitro in which excess magnesium inhibits calcification of osteoid.[10] Maternal bone mineral abnormalities after long-term $MgSO_4$ therapy, however, have not been reported.

The cumulative effects of long-term $MgSO_4$ therapy

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may differ quantitatively from the effects of short-term therapy. We hypothesize that because maternal calcium homeostasis may not adapt during long-term $MgSO_4$ therapy, metabolic perturbations could be detrimental to the mother. The objective of our study was to measure indices of calcium homeostasis and bone mineralization in hospitalized pregnant women in preterm labor receiving long-term $MgSO_4$ therapy.

Material and methods

Subject population. Pregnant women admitted to the labor and delivery units at St. Luke's Episcopal Hospital and The Methodist Hospital between 24 and 32 weeks' gestation were enrolled after informed written consent was obtained by one of the investigators. The $MgSO_4$ group comprised women in preterm labor in whom intravenous therapy with magnesium sulfate for more than 1 week and strict bed rest were anticipated. A control population, in which $MgSO_4$ was not used, comprised similar women in whom strict bed rest was anticipated for the obstetric indication of either placenta previa or preterm labor. Subjects were between 18 and 40 years of age; were free of systemic illness, chorioamnionitis, and medications known to affect calcium metabolism (e.g., diuretics, heparin, or anticonvulsants); and were not known to have fetal malformations or growth retardation. Groups also were matched for race and multiple gestations. The protocol was modified to exclude postpartum measurements of bone indices for subjects if $MgSO_4$ therapy was discontinued >1 week before delivery or if delivery occurred beyond 34 weeks' gestation. The protocol was approved by the institutional review boards for human research at the respective institutions.

The choice of tocolytic therapy was made by the attending obstetrician. $MgSO_4$ was administered as an intravenous loading dose of 6 gm over 30 minutes, followed by a maintenance dose of 2 gm/hr. The $MgSO_4$ infusion was titrated from 1.5 to 3.5 gm/hr, depending on uterine activity.

Study design. There were three sections of the study: measurements of indices of calcium homeostasis in serum, urine, and bone. Baseline (pre-$MgSO_4$ therapy or at enrollment in the control group) and weekly blood samples were obtained for analyses of magnesium, total and ionized calcium, phosphorus, parathyroid hormone, osteocalcin, alkaline phosphatase activity, albumin, and vitamin D metabolites. At the end of each week, urine was collected for 24 hours for the analyses of Mg, Ca, P, zinc, copper, hydroxyproline, and creatinine. Bone mineral content of the nondominant distal radius was measured 1 week post partum and at term-equivalent age (approximately 2 to 3 months post partum). Subjects maintained an activity diary throughout the hospitalization to assess the percentage of each day spent at bed rest.

Analytic methods. Magnesium, Ca, P, and creatinine in the serum and urine and alkaline phosphatase activity and albumin in serum were measured with colorimetric assays of the Cobas Fara autoanalyzer (Roche Diagnostics, Nutley, N.J.). Serum ionized calcium was measured by an ion selective electrode (Radiometer Corp., Copenhagen). Urinary Zn and Cu were determined by atomic absorption spectrophotometry. Urinary hydroxyproline was determined with a Beckman System 6300 amino acid analyzer (Beckman Instruments, Inc., Palo Alto, Calif.).[11] Radioimmunoassays were used to measure serum midmolecule parathyroid hormone and osteocalcin (INCSTAR, Stillwater, Minn.), 25-hydroxyvitamin D (Nichols Institute, San Juan Capistrano, Calif.) and 1,25-dihydroxyvitamin D.[12] Bone mineral content of the nondominant distal third of the radius was measured by single-photon absorptiometry (SP-2 scanner, Lunar Radiation Corp., Madison, Wis.).

Statistical analyses. Subject characteristics were analyzed by Student t test. The χ^2 and Fisher exact analyses were used to test dichotomous variables. The data were analyzed by repeated measures analysis of variance. Although some subjects were studied beyond 4 weeks, because of the dropout rate, only the first 3 weeks' data were tabulated. Average values for weekly (weeks 1 to 3) samples were reported when no changes were observed in repeated measurements over time. Regression analysis was used to investigate relationships between dependent and independent variables. The sample size was selected to detect a difference of 1 SD in outcome measures with a type I error of 0.05 and a power of 0.80. Data are tabulated as mean ± SD unless indicated otherwise.

Results

The characteristics of the 20 subjects in the $MgSO_4$ group and the 19 subjects in the control group were similar with respect to maternal age, parity, number of multiple gestations, racial distribution, length of gestation at enrollment and delivery, percentage of time at strict bed rest, and maternal anthropometric indices at delivery (Table I). Antenatal glucocorticoid therapy was used in 80% of the $MgSO_4$ group and 74% of the control group ($p > 0.10$). Premature rupture of membranes was documented in 50% of the $MgSO_4$ group and 32% of the control group ($p > 0.1$). Therapy with β-sympathomimetic agents was administered to 65% of $MgSO_4$ and 42% of control subjects ($p > 0.05$). The groups were distributed similarly with respect to season of enrollment. The mean duration of $MgSO_4$ therapy was 26 ± 14 days with a range of 8 to 63 days. The mean cumulative $MgSO_4$ dosage was 1405 ± 963 gm (range 342 to 4542 gm). The control group was hospitalized for 21 ± 14 days (range 7 to 56 days). The incidence and duration of lactation were similar be-

tween groups (median 5.5 weeks, range 0 to 36 weeks). Because of the study design and the right of subject refusal, some women participated in only two of the three sections of the study. Subject characteristics were similar between groups and resembled those of subgroups from the overall study population in whom serum (n = 32), urine (n = 27), or bone (n = 26) indices were measured.

Baseline serum Mg, total Ca, and P concentrations were similar between groups (Fig. 1). Serum Mg concentrations increased from baseline nearly threefold in the $MgSO_4$ group and were significantly greater in the $MgSO_4$ group than in the control group during 3 weeks of study ($p < 0.001$ by repeated measures analysis of variance). Serum concentrations of P increased and total Ca decreased in the $MgSO_4$ group from baseline, and each differed significantly between groups during 3 weeks of study ($p < 0.001$ by repeated measures analysis of variance for each). Serum concentrations of ionized Ca were similar at baseline (average 5.04 ± 0.08 mg/dl) in both groups. When measured at week 2 of study, serum concentrations of ionized Ca declined significantly in the $MgSO_4$ group (4.16 ± 0.44 mg/dl, $p = 0.006$) but remained unchanged in the control group. The serum total and ionized Ca concentrations were correlated ($r = 0.94$, $p < 0.001$); the decline in total Ca was attributed to the change in the ionized Ca fraction.

Serum parathyroid hormone concentrations were similar between groups at baseline. Serum parathyroid hormone concentrations increased from baseline in the $MgSO_4$ group and were significantly greater than those of the control group during the 3-week study ($p < 0.001$ by repeated measures analysis of variance, Fig. 2). Changes in parathyroid hormone concentrations from baseline were negatively correlated with changes in serum Ca concentrations ($r = -0.57$, $p = 0.002$); correlations with changes in serum Mg concentrations ($r = 0.31$, $p = 0.1$) did not reach statistical significance at the 0.05 level.

Serum osteocalcin concentrations were similar between groups; both groups had an increase from baseline (0.9 ± 1.5) to week 3 (1.2 ± 1.5 ng/ml, $p = 0.03$). Serum concentrations at baseline and during the 3-week study were similar between groups and over time for albumin (average of baseline and weekly concentrations, 3.0 ± 0.3 gm/dl), alkaline phosphatase activity (117 ± 56 IU/L), 25-hydroxyvitamin D (30 ± 16 ng/ml), and 1,25-dihydroxyvitamin D (56 ± 15 pg/ml). Serum concentrations of 25-hydroxyvitamin D did not change during the study interval but manifested significant seasonal variability. Greater concentrations of 25-hydroxyvitamin D were observed during summer (44 ± 11 ng/ml) than winter (22 ± 13 ng/ml, $p = 0.002$) months.

The 24-hour urinary excretion of Mg, Ca, P, Zn, Cu, hydroxyproline, and creatinine did not change during the 3-week study. The average value derived from the three weekly measurements of urinary Mg, Ca, and Cu output differed significantly between groups (Table II). There was nearly a threefold greater urinary output of Mg and Ca in the $MgSO_4$ group than in the control group. Creatinine clearance was similar between $MgSO_4$ and control groups (126 ± 42 vs 136 ± 35 ml/min, respectively). The percentage tubular reabsorption of P differed significantly between $MgSO_4$ and control groups (91% ± 4% vs 87% ± 4%, respectively, $p = 0.02$). The percent Ca reabsorption also differed significantly between $MgSO_4$ and control groups (93% ± 2% vs 98% ± 1%, respectively, $p < 0.001$). Serum osteocalcin concentrations correlated significantly with urinary copper output ($r = 0.40$, $p = 0.05$). The Mg dose correlated significantly with urinary output of Mg ($r = 0.51$, $p = 0.01$) and Ca ($r = 0.66$, $p = 0.001$). Urinary Ca output significantly correlated with urinary output of Mg ($r = 0.82$, $p < 0.001$), Ca ($r = 0.57$, $p = 0.002$), and P ($r = 0.49$, $p = 0.01$).

Bone density of the distal radius at 1 week post partum (701 ± 55 vs 675 ± 53 mg/cm²) was similar between $MgSO_4$ (n = 15) and control (n = 11) groups, respectively. No differences between groups were detected in either bone width or absolute bone mineral content at the site of measurement. Bone density was not correlated with body weight, height, race, age, or duration of hospitalization. Bone width was correlated with body weight ($r = 0.54$, $p = 0.005$) and height ($r = 0.43$, $p = 0.03$).

Because of the referral nature of the study population, traveling distance enabled only 15 women to return for follow-up measurements of bone mineralization at term-equivalent age. When measured at 11 ± 3 weeks post partum (42 ± 3 postmenstrual weeks), bone density was similar between groups. Repeated measures analysis of variance for bone density identified a sig-

Table I. Characteristics of subjects

Characteristic	*Group*	
	$MgSO_4$ (n = 20)	*Control (n = 19)*
Age (yr)	29 ± 6	29 ± 5
Median parity (range)	1 (0-2)	1 (0-3)
Multiple gestation (No.)	5	3
Race		
White	15	15
Black	3	2
Hispanic	2	2
Entry gestation (wk)	28 ± 2	29 ± 3
Delivery gestation (wk)	33 ± 3	33 ± 3
Time on bed rest (%)	98 ± 3	98 ± 4
Body weight at delivery (kg)	64 ± 6	71 ± 12
Maternal height (cm)	164 ± 8	162 ± 8

Values are mean ± SD.

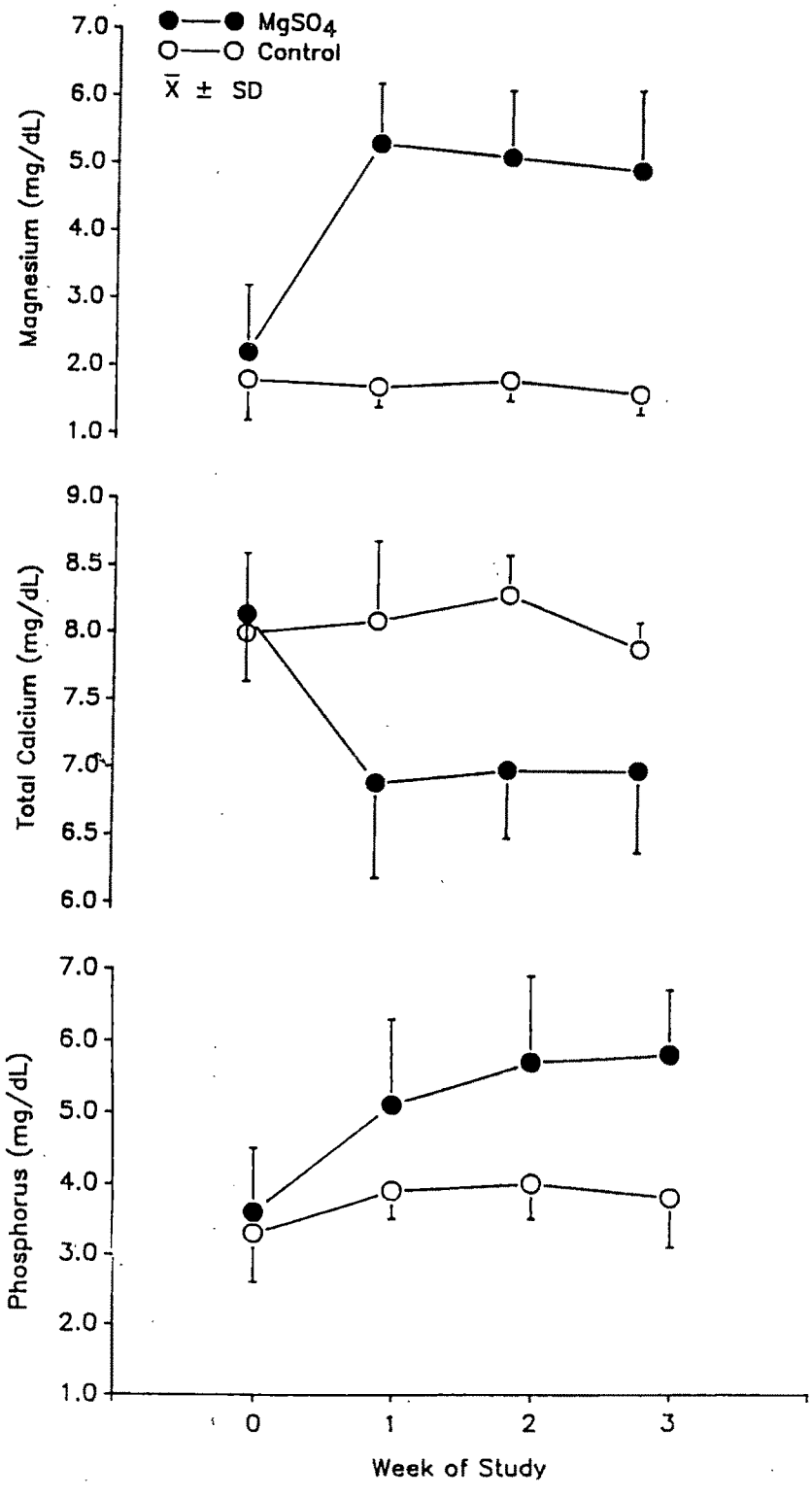


Fig. 1. Serum Mg, total Ca, and P in $MgSO_4$ and control groups at baseline (time = 0) and during 3 weeks (weeks 1 to 3) of study. Significant differences ($p < 0.001$) between groups, determined by repeated measures analysis of variance, were observed during 3-week study but not at baseline.

nificant interaction effect between study group and time. In the postpartum interval there was a decline in the bone density in the $MgSO_4$ group ($n = 7$) and a rise in bone density in the control group ($n = 8$); this difference between groups was significant ($p = 0.03$).

With respect to either demographic characteristics or bone indices at 1 week post partum, the 15 women who

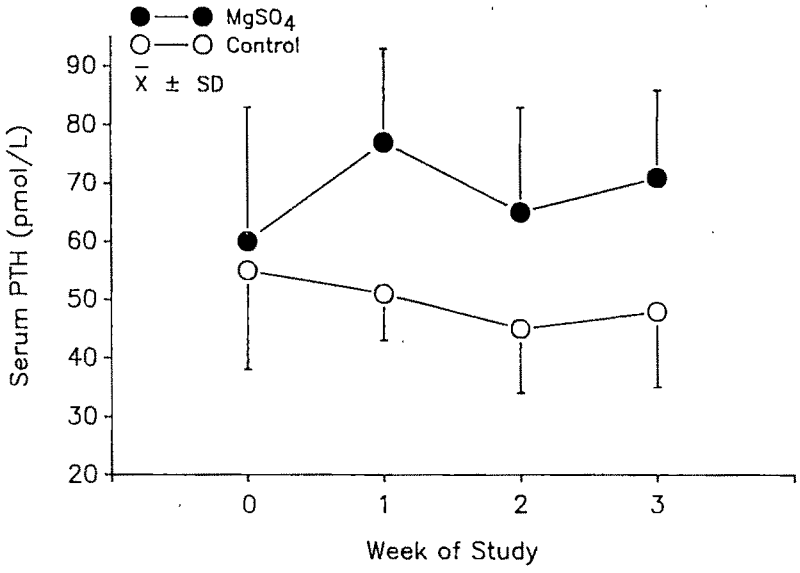


Fig. 2. Serum parathyroid hormone *(PTH)* in $MgSO_4$ and control groups at baseline (time = 0) and during 3 weeks (weeks 1 to 3) of study. Significant differences ($p < 0.001$) between groups, determined by repeated measures analysis of variance, were observed during 3-week study but not at baseline.

returned for follow-up did not differ from the overall study group. Overall differences that had been observed between groups in serum and urine indices also were found in the subgroups.

Comment

Our study demonstrates that calcium homeostasis in pregnancy is affected by long-term intravenous $MgSO_4$ therapy. The alterations in serum and urine Mg and Ca parallel those reported after short-term (24 to 48 hours) $MgSO_4$ therapy.[3-5] Alterations in the latter case, however, are reversible after discontinuation of the $MgSO_4$ therapy.[3, 4] Serum concentrations of Ca decreased and those of P and parathyroid hormone increased and remained at the altered levels for the duration of the study. Urinary Mg, Ca, and Cu excretion remained significantly greater in the $MgSO_4$ group compared with the control group throughout the study interval. We did not examine these variables after the therapy was discontinued. During therapy, however, we observed no changes in the measured indices to indicate an adaptation (i.e., via diet or altered gastrointestinal nutrient absorption) to the therapy.[13]

The mechanism by which $MgSO_4$ therapy results in a decline in serum Ca concentration is unclear. Magnesium may inhibit or compete for common Ca reabsorptive sites in the loop of Henle.[14, 15] Our data demonstrated that the percent filtered Ca that was reabsorbed was significantly lower in the $MgSO_4$ group (93%) than in the control group (98%). We suggest therefore that the decline in serum Ca was a direct response to the elevated serum Mg and its action at the nephron. Similarly, the elevated serum P observed in the $MgSO_4$ group also may have resulted from hypermagnesemia and its effect on the reabsorption of P. We observed a greater percent tubular reabsorption of P in the $MgSO_4$ group (91%) than in the control group (87%), thus potentially explaining the greater serum P concentrations in the $MgSO_4$ group.

Table II. Average urinary metabolite excretion (milligrams per 24 hours) during first 3 weeks of study*

	Group	
	$MgSO_4$ (n = 14)	*Control (n = 13)*
Magnesium†	245 ± 95‡	94 ± 68
Calcium†	844 ± 313	343 ± 145
Phosphorus	854 ± 291	903 ± 315
Copper§	3.4 ± 1.0	2.4 ± 1.1
Zinc	7.6 ± 3.3	9.1 ± 7.3
Hydroxyproline	54 ± 25	44 ± 14
Creatinine	1103 ± 177	1224 ± 396

*There were no differences over time.
†$p < 0.001$.
‡Mean ± SD.
§$p = 0.02$.

Our data do not support a role for parathyroid hormone in the genesis of hypocalcemia that occurs with hypermagnesemia. We found significantly increased parathyroid hormone secretion in the $MgSO_4$ group, which is contrary to other reports of short-term $MgSO_4$ therapy in which parathyroid hormone secretion was suppressed.[5, 16] Cruikshank et al.,[3] however, demonstrated that in women with preeclampsia the serum parathyroid hormone concentrations increase promptly and substantially in association with Mg-

induced decreases in serum ionized Ca concentrations. They commented that parathyroid hormone secretion is a protective response without which hypocalcemia would be manifested to a greater degree. Our data supported the latter contention. We found a significant correlation between the change in serum parathyroid hormone concentration and the change in serum Ca but not the change in serum Mg concentrations.

The urinary output of Ca and Mg in the $MgSO_4$ group was quantitatively similar to that reported during short-term therapy. Several investigations have demonstrated that urinary Mg excretion approximates 75% of the infused Mg dose during short-term studies and 99% if excretion is monitored beyond the infusion interval.[17,18] We observed that the quantity of Mg excreted was less than that infused. However, we monitored neither urinary excretion beyond the therapy interval nor the fetoplacental-amniotic fluid unit during therapy. We speculate, however, that a portion of the infused Mg may have been stored or deposited in the body.[13] Mg complexes with hydroxyapatite crystals and bone contents correlate positively with serum Mg concentrations.[13] We also considered that other divalent cations might be displaced by the presumed Mg deposition in bone. Approximately 30% of total body stores of zinc and 19% of copper are found in bone.[19] The explanation of the greater urinary losses of Cu in the $MgSO_4$ group is unknown. Further investigation is warranted.

The urinary losses of Ca, however, are a great concern. An increased daily loss of approximately 0.5 gm of Ca (difference between $MgSO_4$ and control groups) potentially might affect skeletal composition. If we assume that the total body Ca content is 900 gm in a young adult woman,[20] our subjects may have lost as much as 4% of total body Ca during a 60-day interval. Dietary intake and enhanced gastrointestinal absorption certainly restore a proportion of the Ca. Nevertheless, urinary losses of Ca approached the Recommended Dietary Allowance for Ca in pregnancy, 1,200 mg/day.[21] We speculate that usual Ca intakes during pregnancy would be inadequate to meet Ca needs and restore the Ca lost as a result of $MgSO_4$ therapy.

Osteocalcin is a sensitive marker for bone formation and bone remodeling. The serum concentration of osteocalcin rises in conditions characterized by high remodeling and declines when bone turnover is low.[22-24] We observed similar low serum concentrations of osteocalcin in both study groups. Although the reason for the modest elevation in serum osteocalcin in both groups is unclear, it may be associated with increased bone turnover in later pregnancy.[24] The relationship between osteocalcin and Mg has been studied.[25,26] In animal models serum osteocalcin concentrations increase markedly during $MgSO_4$ infusion. Those observations are consistent with studies in vitro demonstrating Mg displacement of osteocalcin from bone.[26] Whether similar Mg activity occurs during human pregnancy is unknown. We found no association between $MgSO_4$ and osteocalcin.

The similarities between groups in bone mineralization values 1 week post partum is paradoxic considering the large losses of Ca in urine in the $MgSO_4$ group. We speculate, however, that Mg displacement of other divalent cations may not have affected the measured bone density. We suggest that Mg may have been deposited in bone but did not affect bone turnover as evidenced by the lack of differences between groups in serum osteocalcin, alkaline phosphatase activity, or urinary hydroxyproline.

The apparent decline in bone density in the $MgSO_4$ subgroup from 1 to 11 weeks post partum was surprising but consistent with a slow loss of Mg deposited in bone during hypermagnesemia.[13] We sought other explanations for the differences in the rate at which bone density changed. We knew that subgroups had similar histories of lactation and medication usage. The observations should be interpreted cautiously, however, because of the small sample of women who were able to return for follow-up. Further evaluation of bone density changes should be undertaken.

Long-term $MgSO_4$ therapy produced changes in Ca homeostasis that were similar to those after short-term therapy. The duration of therapy did not appear to affect the magnitude of the changes in serum and urine indices; we observed no adaptation in these indices during long-term therapy. Nevertheless, the hypocalcemia was "stabilized" by parathyroid hormone activity on bone. Therefore $MgSO_4$ therapy may prevent extreme preterm delivery, but further evaluation of its effects, especially pertaining to bone mineralization, is warranted.

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Nifedipine for treatment of preterm labor: A historic prospective study

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OBJECTIVES: The purpose of this study was to ascertain whether adverse fetal and/or neonatal effects occurred during nifedipine treatment of preterm labor and to assess maternal tolerance of nifedipine therapy in patients intolerant of a β-sympathomimetic agent

STUDY DESIGN: We undertook historic prospective review of medical records of 102 women admitted to an antepartum ward for treatment of preterm labor who received nifedipine. Data were collected regarding maternal side effects, fetal surveillance, and neonatal outcome.

RESULTS: The number and severity of reported maternal side effects were significantly reduced when patients were switched from terbutaline to nifedipine. No discontinuance of nifedipine occurred because of maternal side effects. Fetal surveillance testing and neonatal outcome data failed to reveal deleterious in utero effects of nifedipine.

CONCLUSIONS: Nifedipine was a well-tolerated and safe tocolytic in this population and warrants further investigation. (AM J OBSTET GYNECOL 1992;167:52-6.)

Key words: Nifedipine, β-sympathomimetic agents, tocolysis, preterm labor

Prematurity and low birth weight continue to be critical public health problems. In spite of marked reductions in infant mortality rates in the United States, the incidence of preterm birth has remained essentially unchanged in the past decade, at 8% to 10% of all births.[1] Tocolysis, the pharmacologic inhibition of uterine contractions, is currently the principal preterm birth preventive measure and will remain so until the etiology of preterm labor is better understood.

The tocolytic drugs most frequently used in the United States are the β-sympathomimetic agents, usually terbutaline or Food and Drug Administration–approved ritodrine.[2] Intravenous administration of these agents has a rapid tocolytic effect, and oral administration may be used for maintenance therapy. The β-sympathomimetic drugs, however, have significant and potentially serious maternal and fetal side effects (e.g., pulmonary edema, cardiac ischemia, and fetal cardiotoxicity[2, 3]). Less serious but more prevalent are the expected systemic side effects of tocolytic doses of β-adrenergic agonists, such as tachycardia, tremors, anxiety, palpitations, and insomnia.[4] Maternal intolerance of these side effects often requires reduction in dosage or discontinuance of therapy.[5]

Nifedipine, a calcium channel-blocking agent, has emerged as a potentially safer and better tolerated tocolytic agent.[6-8] Nifedipine inhibits smooth muscle contraction by impeding the flow of calcium across the muscle cell membrane.[9] Successful treatment of preterm labor with nifedipine has been reported.[10, 11] Maternal side effects were minimal in these 11 patients, consisting of mild transient headaches and a flushing sensation. In the single randomized trial published to date, nifedipine was found to be more efficacious and better tolerated than ritodrine.[6] Fetal surveillance during nifedipine therapy and neonatal outcome assessments revealed no evidence of adverse fetal effects in any of these studies.

The fetal safety of nifedipine was questioned, however, in several animal studies reported in the mid-1980s. In pregnant sheep and rhesus monkeys, where a continuous infusion of nicardipine* was administered, significant decreases in fetal arterial Po_2 and pH were found.[12, 13] To date, there have been no reports of adverse fetal effects of nifedipine in humans.

In 1987 nifedipine was introduced as an alternative tocolytic agent for Kaiser Permanente patients at St. Joseph Hospital. Initially, nifedipine was reserved for women who exhibited intolerance to therapeutic doses of other tocolytics, usually terbutaline. Close fetal surveillance was maintained after nifedipine therapy was

*Nicardipine is the intravenous analog of nifedipine.

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begun to detect possible adverse fetal effects. Nifedipine appeared to be an effective tocolytic agent; it was well tolerated by patients and adverse fetal effects were not observed. However, in light of concern over fetal safety raised by some animal studies, a systematic review of all patients treated with nifedipine was undertaken. The objectives of this historic prospective study were as follows: (1) to ascertain whether adverse effects occurred among mothers and/or fetuses treated with nifedipine and (2) to assess nifedipine's usefulness in treating women in preterm labor who were intolerant of other tocolytics.

Material and methods

Study population. Medical records were reviewed for all women admitted to the St. Joseph Hospital antepartum ward with the diagnosis of preterm labor from Jan. 3, 1988, through March 31, 1989. Records of women admitted with conditions associated with preterm labor (such as pyelonephritis) were also reviewed. Medication records, physicians' orders, and discharge summaries were examined to identify patients who had ever received nifedipine. Subsequent hospital admissions, outpatient prenatal charts, and labor and delivery records were then reviewed for all patients identified as having been treated with nifedipine. Informed consent for the experimental usage of nifedipine was documented in all identified charts.

Patients with six or more regular uterine contractions in 1 hour who were admitted to the hospital for tocolysis were included in the study. Documentation of cervical change varied widely between clinicians and thus was not used as a criterion for inclusion.

Data collection. Data were abstracted from the medical records of all patients who had received nifedipine. The physician's progress notes and the nurse's notes were reviewed for reports of maternal side effects. Days of treatment were calculated from the first day of administration until the day of discontinuance. Clinical reasons given for discontinuing, changing, or adding tocolytics were coded and recorded. Prenatal records were reviewed for outpatient fetal surveillance data. The delivery record was the source of neonatal data. Gestational age was based on the obstetric estimate used in the medical record. The infant's medical record was reviewed only if an adverse outcome was reported.

Limitations. The reviewers were not blinded to the study objectives, but the first 30 charts (29.4%) were examined by two reviewers to establish interobserver reliability. Information regarding maternal side effects came from medical records only rather than directly from the patient. Thus this information is dependent on accurate recording in the nurse's and physician's notes. In general, the nurse's notes were the most informative. Their charting format would frequently directly quote the patient's statements regarding subjective symptoms. More serious maternal side effects, such as chest pain, tended to be more thoroughly documented than more minor complaints, and thus our methods were probably more sensitive for the severe side effects.

In this descriptive study the question of tocolytic efficacy was not addressed. Further, the lack of a comparison group treated with terbutaline only limits our conclusions.

Table I. Medication experiences of study population ($N = 102$)

Group	*No.*	*%*
Group 1. Terbutaline switched to nifedipine	51	50
Group 2. Terbutaline and nifedipine alternating	29	28.4
Group 3. Nifedipine only	11	10.8
Group 4. Nifedipine combined with other tocolytic*	11	10.8
TOTAL	102	100

*Magnesium sulfate or indomethacin.

Results

A total of 119 patients were identified as having been treated with nifedipine for preterm labor during the 15-month study period. Seventeen patients (14.3%) were excluded from analysis because of incomplete information. Primigravid women constituted 33.3% of the study group, and the overall mean maternal age was 28 years.

Pregnancy complications in addition to preterm labor were experienced by 41 patients (40% of the study population). Twin gestations made up 18% of the entire study population but accounted for 38.3% of patients with complications. Second-trimester bleeding, usually placenta previa, was the next most frequent complication (21.3%), followed by maternal diabetes (8.4%).

Whereas there were varied patterns of tocolytic administration within the study population, four groups could be distinguished for descriptive purposes (Table I). Patients treated with terbutaline initially and then subsequently switched to nifedipine composed the largest group (group 1). Approximately 28% of the patients were treated with alternating doses of terbutaline and nifedipine (group 2), 11% received nifedipine alone (group 3), and 11% received nifedipine combined with another tocolytic agent* (group 4).

Of the 51 patients in group 1, 40 (78%) were switched to nifedipine because of adverse side effects of terbutaline. Table II compares the side effects reported by this group while they were receiving each of these tocolytics. Chest pain was experienced by 50% of the

*Magnesium sulfate or indomethacin.

Table II. Side effects reported by patients switched to nifedipine because of intolerance of terbutaline (n = 40)

Side effect	*Terbutaline** No.	%	*Nifedipine†* No.	%	$p < 0.05$‡
Chest pain	20	50	1	2.5	Yes
Dyspnea	15	37.5	1	2.5	Yes
Palpitations	15	37.5	0	0	Yes
Anxiety	15	37.5	0	0	Yes
Tremors	8	20	0	0	Yes
Ischemic electrocardiographic changes	5	12.5	0	0	Yes
Tachycardia (heart rate >120 beats/min)	5	12.5	0	0	Yes
Nausea and vomiting	3	7.5	2	5.0	No
Headache	1	2.5	7	17.5	Yes
Constipation	0	0	3	7.5	—§
Dizziness	0	0	1	2.5	—§
Flushing	0	0	1	2.5	—§
Fatigue	0	0	1	2.5	—§
TOTAL	82‖		17¶		

*Side effects of terbutaline leading to change to nifedipine.

†Side effects reported after change to nifedipine.

‡The Sign test (True Epistat, 3rd ed. Tracy L. Gustafson) was used to compare side effects of these two tocolytic agents.

§Matched pairs of insufficient size for analysis.

‖Twelve patients (30%) reported a single side effect, whereas 28 patients (70%) reported two or more side effects.

¶More than one side effect was reported by two patients (5%), whereas 25 patients (62.5%) reported experiencing no side effects.

terbutaline group, with 12.5% showing ischemic electrocardiographic changes. Dyspnea (37.5%) and palpitations (37.5%) were the next most frequent side effects leading to discontinuance of terbutaline. Significant reductions in all of the side effects experienced with terbutaline occurred after a switch to nifedipine, except for nausea and vomiting, which remained about the same. While the patients were on a regimen of nifedipine, headache was the leading reported side effect (17.5%), and 62.5% reported no side effects.

Similar reductions in the number and severity of reported side effects are seen when all study patients treated with terbutaline and nifedipine are evaluated (Table III). Palpitations (41.2%), chest pain (39.2%), and dyspnea (29.4%) were again the most frequently reported side effects with terbutaline. Patients treated with nifedipine alone, as either the primary or secondary tocolytic, reported fewer side effects (30 vs 94), with nearly 52% reporting no side effects. Headache (19.4%), flushing (8.1%), and nausea and vomiting (6.5%) were the most common side effects reported in the nifedipine group. In no instance did maternal side effects necessitate discontinuance of nifedipine.

Table III. Comparison of side effects

Side effect	*Terbutaline** (n = 51) No.	%	*Nifedipine†* (n = 62) No.	%
Palpitations	21	41.2	0	0
Chest pain	20	39.2	1	1.6
Dyspnea	15	29.4	0	0
Anxiety	14	27.5	0	0
Tremors	10	19.6	0	0
Ischemic electrocardiographic changes	5	9.8	0	0
Tachycardia (heart rate >120 beats/min)	5	9.8	0	0
Nausea and vomiting	3	5.9	4	6.5
Headache	1	2.0	12	19.4
Constipation	0	0	3	4.8
Dizziness	0	0	3	4.8
Flushing	0	0	5	8.1
Fatigue	0	0	1	1.6
TOTAL	94‡		30§	

*Side effects reported while receiving terbutaline.

†Side effects reported while receiving nifedipine.

‡Thirty patients (58.8%) had two or more side effects.

§Thirty-two patients (51.6%) reported no side effects.

Fetal surveillance during tocolytic treatment, consisting of a nonstress test and/or a biophysical profile, was performed at least once in 79% of the singleton gestations and in 100% of the twin gestations. Of the 265 total surveillance tests performed, only one test result was abnormal. This nonreactive nonstress test was followed by a normal biophysical profile, and there was a normal neonatal outcome. The number of surveillance tests performed was directly related to the duration of tocolysis, with more tests performed on fetuses with longer exposures to nifedipine.

Interval fetal growth assessments were performed by ultrasonography on 65% of those exposed to nifedipine in utero, with adequate growth demonstrated in 92.3%. Of the 15 twin gestations monitored by serial ultrasonography, two sets of twins showed mild and one set showed moderate discordant growth; tocolytic therapy was discontinued. Three singleton gestations showed evidence of intrauterine growth retardation (IUGR). Maternal hypertension was present in one case, and there were known fetal anomalies in another, whereas the third fetus had no known risk factors for IUGR. Therefore five of the six fetuses with inadequate growth during tocolysis had other possible causative factors for IUGR.

Although there was no control group that had not received a tocolytic agent, pregnancies were extended 4.9 weeks from the time of preterm labor diagnosis. The mean estimated gestational age was 30.7 weeks at initiation of tocolysis and 35.6 weeks at discontinuance

of therapy. Overall, nearly 60% of the study group were delivered at term. The cesarean section rate for the study group was 26.5%, with only two procedures (2%) performed for fetal distress. Twin gestations had a 50% cesarean section rate in our study group.

Table IV contains neonatal outcome data for the entire study group. Mean birth weight was 2582 gm and mean gestational age was 36.8 weeks. Five infants (4.2%) had 5-minute Apgar scores <7, although only one had a venous cord pH <7.20. Assisted ventilation in the delivery room was required for 13 infants (10.8%). A total of 54 infants (45%) were taken from the delivery room to the neonatal intensive care unit, although this includes those neonates taken for observation only and then sent to the newborn nursery, a relatively standard procedure for preterm infants in this hospital. The previously described infant with congenital anomalies and IUGR was the only neonatal death in the study group. This infant died at 8 days of age from congenital heart and renal anomalies.

Of the 120 infants exposed to nifedipine, six infants (5%) were in the lst to 10th percentile of weight for age and thus met clinical criteria for IUGR. There were no infants in the severely growth-retarded (<1% weight for age) category. Overall, the weight percentiles were normally distributed.

Table IV. Outcome assessment for all neonates exposed to nifedipine in utero (n = 120)

Birth weight (gm)	
Mean ± SD	2582 ± 512
Range	1000-4140
Gestational age (wk)	
Mean ± SD	36.8 ± 2.7
Range	29.0-41.9
Estimated gestational age at birth >37 wk (No.)	58 (56.9%)
Apgar score <7 at 5 min (No.)	5† (4.2%)
Assisted ventilation in delivery room‡ (No.)	13 (10.8%)
Neonatal intensive care unit§ (No.)	54 (45%)
Neonatal death (No.)	1‖ (0.8%)

*Includes 84 singletons and 36 twins.

†All had venous cord gas values determined; only one value was <7.20.

‡Bag-mask or intubation required.

§Taken to neonatal intensive care unit from delivery room (includes those taken for observation only).

‖Known congenital anomalies at initiation of tocolysis (dilated viscera, abnormal spine, intraabdominal calcifications) but with normal karyotype. Cardiac and renal anomalies diagnosed at birth were incompatible with life.

Comment

Our historic prospective study of 102 patients in preterm labor treated with nifedipine found that nifedipine is a better tolerated and safer tocolytic agent than terbutaline. Patients experiencing potentially serious side effects while receiving terbutaline (chest pain, tachycardia, ischemic electrocardiographic changes) had significant abatement of symptoms after they were switched to nifedipine and, in many cases, reported no subsequent side effects. The side effects reported most frequently with nifedipine were headache, constipation, and nausea, a dramatic contrast to those reported by patients receiving terbutaline. When one considers that the potentially fatal cardiorespiratory complications seen with β-sympathomimetic therapy are often preceded by side effects such as tachycardia, dyspnea, and chest pain, the difference in side effect profiles between these two agents becomes even more meaningful.

Clearly, the fetal effects of nifedipine have been the major concern regarding its use for preterm labor. The only alleged presumptive danger to the fetus has been reported in animal studies of uterine perfusion and fetal oxygenation.[14,15] In this study there were 265 fetal surveillance tests performed during nifedipine therapy, with only one abnormal test. Because of our limited sample size, there may have been insufficient power to detect adverse fetal effects of nifedipine. However, the absence of evidence indicating hypoperfusion or deoxygenation of nifedipine-exposed fetuses was reassuring.

Ritodrine was approved for use in treating preterm labor in 1980 and has since become a first-line tocolytic agent. In the ensuing decade negligible improvement was noted in the rate of preterm birth, yet maternal, fetal, and neonatal morbidity and mortality attributable to ritodrine and other β-sympathomimetic agents have been recorded. The psychotropic changes induced by the β-sympathomimetic agents, such as anxiety and insomnia, cause enormous maternal discomfort and are the exact opposite of those desired—rest and relaxation for prolonging pregnancy. In our study nifedipine was used as a tocolytic agent with no maternal morbidity, minimal maternal discomfort, and no demonstrable deleterious fetal or neonatal effects.

A randomized prospective trial of nifedipine is needed to compare its efficacy with that of other standard tocolytic agents. In such a study close fetal surveillance and detailed neonatal and infant follow-up will be important. Calcium channel blockers show great promise as tocolytic agents for both mother and fetus.

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Nonimmune hydrops fetalis associated with maternal infection with syphilis

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Intrauterine infection with syphilis was diagnosed by reactive maternal serologic studies, ultrasonographic findings, and exclusion of other causes in three hydropic fetuses at 31, 34, and 35 weeks' gestation. With penicillin therapy and preterm delivery all infants survived through the perinatal period. Intrauterine infection that follows syphilis is a potentially treatable cause of nonimmune hydrops. (AM J OBSTET GYNECOL 1992;167:56-8.)

Key words: Nonimmune hydrops, syphilis, pregnancy

The dramatic increase in the incidence of early syphilis in the United States has lead to a concomitant rise in reported cases of congenital syphilis and nonimmune hydrops associated with maternal infection. Earlier reports of nonimmune hydrops associated with syphilis, which have been limited to developing countries, demonstrate increased perinatal mortality. Chawla et al.[1] in Zimbabwe described three cases with a 100% neonatal mortality, whereas in Singapore, Tan[2] reported on four patients with a 75% mortality. We report three cases of nonimmune hydrops fetalis associated with maternal infection with syphilis managed with an aggressive protocol; all neonates survived.

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Case reports

Case 1. A 21-year-old woman, gravida 2, para 1, with uncertain menstrual dates and no history of syphilis treatment was referred in preterm labor. Antenatal laboratory screen 3 days before admission was significant for a reactive rapid plasma reagin titer of 1:32; she denied previous treatment for syphilis. Penicillin for early latent syphilis was initiated before transfer. The patient's blood type was O negative, and antibody

Table I. Initial newborn laboratory values

	Case 1	*Case 2*	*Case 3*	*Normal*
White blood cell count ($\times 10^9$/L)	95.1	31.4	6.8	5-21
Hemoglobin (gm/dl)	6.0	4.5	12.8	15.5-18.9
Hematocrit (%)	19.9	13.2	39.7	42-56
Platelet count ($\times 10^9$/L)	53	31	67	140-290
Fetal rapid plasma reagin titer	1:16	1:16	1:128	Nonreactive
Fetal microhemagglutination assay for antibodies to *T. pallidum*	Positive	Positive	Positive	Negative
Total protein (mg/dl)	2.2	3.6	4.8	4.6-8.0
Albumin (mg/dl)	0.9	1.8	2.4	3.0-5.5
Immunoglobulin M (mg/dl)	101	172	—	0-25

screen was negative. Ultrasonographic evaluation revealed an intrauterine pregnancy at 31 week's gestation with fetal ascites, marked generalized subcutaneous edema, and hydramnios. There were no other fetal structural abnormalities, and a fetal echocardiogram was normal. On admission a maternal Kleihauer-Betke test was negative, repeat maternal rapid plasma reagin titer was 1:128, and a microhemagglutination assay for antibodies to *Treponema pallidum* was positive. The amniotic fluid lecithin/sphingomyelin ratio was mature. Rapid karyotype from blood obtained by cordocentesis was normal. The patient underwent cesarean section for breech presentation and was delivered of a 3540 gm female infant with Apgar scores of 2 and 3 at 1 and 5 minutes, respectively. Initial laboratory values are summarized in Table I. The neonatal course was significant for persistent pulmonary hypertension treated with hyperventilation alone. The infant remained on a ventilator for 14 days. Radiographic examination revealed metaphyseal rarefaction in all long bones. The infant received 21 days of intravenous penicillin G therapy and was discharged home at 43 days of life.

Case 2. An 18-year-old woman, gravida 2, para 0, abortus 1, at 35 weeks' gestation had fetal ascites and subcutaneous edema noted on routine ultrasonographic evaluation. There were no other structural abnormalities. The maternal blood type was B positive, and an antibody screen was negative. Rapid plasma reagin titer was reactive (1:64) and microhemagglutination assay for antibodies to *T. pallidum* was positive; she denied previous treatment for syphilis. External monitoring of the fetal heart rate was nonreactive with repetitive late decelerations. Because of the gestational age (35 weeks) and the nonreassuring fetal assessment, a primary cesarean section was performed. A 2700 gm female infant was delivered with Apgar scores of 0 and 1 at 1 and 5 minutes, respectively. Paracentesis was performed in the delivery room because of neonatal respiratory compromise from the ascites. Initial neonatal laboratory values are summarized in Table I. The infant required 4 days of mechanical ventilation, received 21 days of intravenous penicillin G, and was discharged home on the twenty-second day of life. Follow-up of the infant at 6 months showed a nonreactive rapid plasma reagin titer.

Case 3. A 21-year-old woman, gravida 1, para 0, was seen at 34 weeks' gestation without prenatal care. Antenatal laboratory screen was significant for a reactive rapid plasma reagin titer (1:32) and a positive microhemagglutination assay for antibodies to *T. pallidum.* Maternal blood type was A positive, and an antibody screen was negative. Fetal ultrasonographic examination revealed ascites, pericardial effusion, generalized subcutaneous edema, and hydramnios. Maternal treatment with penicillin was initiated. An amniocentesis noted a mature lecithin/sphingomyelin ratio (6.8:1). Although initially reassuring, the fetal heart rate tracing became nonreactive, and a contraction stress test was positive. A 2530 gm male infant was delivered by cesarean section with Apgar scores of 8 and 9 at 1 and 5 minutes, respectively. Initial neonatal laboratory values are summarized in Table I. Radiographic examination revealed metaphyseal lucent bands in all long bones. The infant received 12 days of intravenous penicillin G, and the neonatal course was significant for apnea of prematurity necessitating home monitoring. The infant was subsequently discharged at 30 days of age.

Comment

Nonimmune hydrops fetalis is associated with a myriad of maternal and fetal medical conditions. Because of the extremely poor perinatal outcome, management of pregnancies complicated by this disorder should be dictated by specific cause. It is important to consider the feasibility of treatment as determined by the cause before aggressive management is initiated. Congenital syphilis is a potentially treatable cause of nonimmune hydrops and warrants intravenous antibiotic therapy and delivery. If the fetus is extremely premature, high-dose maternal penicillin therapy is indicated until a gestation compatible with survival is achieved.

Current recommendations for the treatment of syphilis complicating pregnancy emphasize the role of penicillin as the drug of choice. Published studies of treatment of maternal infection with nonpenicillin regimens

report unacceptably high failures in preventing congenital syphilis. Patients who report penicillin allergy should undergo testing for confirmation, then desensitization, followed by treatment with penicillin.

The optimal treatment regimen for nonimmune hydrops caused by maternal infection with syphilis has not been determined. Treatment with penicillin, particularly in early syphilis, carries the risk of a maternal Jarisch-Herxheimer reaction, which may further compromise a severely affected fetus. Antenatal testing therefore is critical for monitoring fetal distress in response to both nonimmune hydrops and complications of treatment of syphilis.

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Suppression of 24-hour cholecystokinin secretion by oral contraceptives

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Weight gain is a common side effect during oral contraceptive use. The secretion of the satiety hormone cholecystokinin was recorded for 24 hours in women before and during treatment with oral contraceptives. During treatment serum profiles of cholecystokinin were clearly suppressed, which might be related to increased appetite and weight gain. (Am J Obstet Gynecol 1992;167:58-9.)

Key words: Oral contraceptives, cholecystokinin, secretory profile

Weight gain is a well-known side effect during hormonal contraception, in particular during use of high-dose progestins. The mechanism is incompletely understood, but a stimulation of appetite seems to be an important component. Recent research has emphasized the role of neuropeptides in the regulation of food intake. The gut-brain peptide cholecystokinin is released in response to a meal and has been suggested to be a physiologic satiety factor.[1] In the current study the 24-hour serum profiles of cholecystokinin were monitored in women before and during oral contraceptive use.

Material and methods

Subjects. Complete 24-hour serum profiles of cholecystokinin were established in nine healthy women attending the local health care center for contraceptive counseling. Their mean age was 25 years (range 22 to 31). The women were evaluated before oral contraceptive use (five of them in the follicular phase and four in the luteal phase of the menstrual cycle) and after 2 to 3 months of treatment with monophasic, combined oral contraceptives (30 μg ethinyl estradiol–150 μg desogestrel or 30 μg ethinyl estradiol–150 μg levonorgestrel). The study was approved by the local medical ethics committee.

Blood sampling. Venous blood samples were obtained with a nonthrombogenic blood withdrawal system. A portable, battery-charged excentric pump (Cormed, Medina, N.Y.) was used to obtain continuous sampling from an antecubital vein through heparin-coated polyvinyl chloride tubing (Durascan Medical Products, Odense, Denmark) during normal daily activities. The activity and food intake of subjects were recorded but in no way restricted. Throughout 24 hours collecting tubes were changed at 30-minute intervals, and approximately 5 ml blood was collected in each 30-minute period. Blood samples were centrifuged free of formed elements, and equal amounts of sera were removed and pooled into one sample for every 2-hour period before being stored at −20° C.

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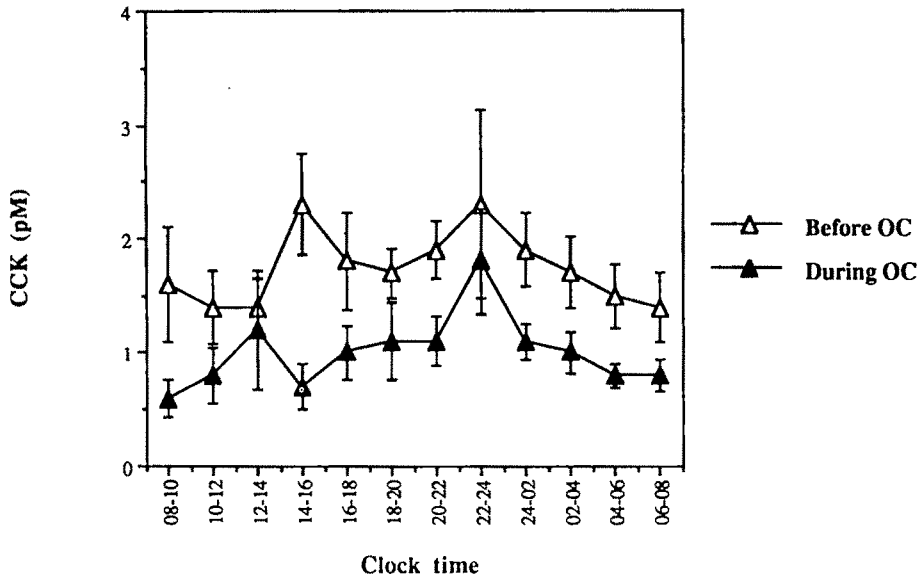


Fig. 1. Serum 24-hour profiles of cholecystokinin *(CCK)* in nine women before and during treatment with oral contraceptives *(OC)*. Values are means ± SEM.

Assay. Before cholecystokinin analysis, sera were extracted and concentrated on C18 Sep-Pak cartridges (Waters Associates, Milford, Mass.). Cholecystokinin was eluted with a mixture of 5 ml 50% acetonitrile and 50% 0.1% acetic acid. Eluted samples were dried and then reconstituted in assay buffer for determination of cholecystokinin with a previously described sensitive and highly specific radioimmunoassay[2] with the modification of using sulfated cholecystokinin-8 as the tracer. Intraassay and interassay coefficients of variations were 4.0% and 10.6%, respectively, and the detection limit of the assay was 2 pmol/L. However, measurement of tenfold lower concentrations of cholecystokinin was made possible by a concentration of the serum samples.

Statistical analysis. Data are presented as means ± SEM. The effect of treatment with oral contraceptives was evaluated with two-factor analysis of variance with repeated measures.

Results and comment

The 24-hour serum profiles of cholecystokinin before and during oral contraceptive use are illustrated in Fig. 1. At all points throughout the 24-hour period cholecystokinin values were lower when women were using oral contraceptives (overall effect $p < 0.001$).

Oral contraceptives are widely used by women for family planning purposes. Still, there is considerable uncertainty about the significance of the many metabolic changes that have been observed during treatment. Weight gain and mood changes have been reported with frequencies varying between 5% and 15% of users. There is increasing evidence that progestins have behavioral effects in women. Progestins may stimulate appetite and in some women cause mood changes, which also are associated with alterations in appetite and food intake. However, the mechanisms of action are not fully explored.

Animal experiments have clearly demonstrated the satiety-inducing effects of peripherally and centrally administered cholecystokinin.[1] Furthermore, the use of recently developed specific cholecystokinin receptor antagonists has provided evidence that endogenous cholecystokinin plays a physiologic role in food intake regulation. Cholecystokinin suppresses food intake not only in experimental animals but also in humans. Recent studies have shown disturbances in meal-related cholecystokinin secretion in eating and affective disorders.[1]

The current results clearly demonstrate a suppression of 24-hour serum levels of cholecystokinin during oral contraception. This might well relate to increased appetite and weight gain in individual women. Further studies are clearly indicated to evaluate the significance and mechanism behind this effect.

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Delayed endometrial maturation induced by daily administration of the antiprogestin RU 486: A potential new contraceptive strategy

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OBJECTIVE: Our objective was to determine if a progesterone antagonist might interdict the development of a secretory endometrium.

STUDY DESIGN: Eleven normally cycling women not at risk for pregnancy received RU 486 (1 mg/day orally) or placebo throughout one menstrual cycle in a randomized, double-blind, crossover fashion. Estradiol, progesterone, and placental protein 14 were measured every 3 days; luteinizing hormone was measured until the midcycle surge was detected. An endometrial biopsy was performed on luteal phase day 7 to 9 and interpreted with Noyes' criteria. Differences between treatment groups were analyzed by the Student *t* test.

RESULTS: RU 486 delayed ovulation, retarded endometrial maturation, and reduced peak levels of placental protein 14 without affecting gonadal steroid production. The abnormalities in endometrial morphology and function are similar to those seen in infertile women with luteal phase defects.

CONCLUSION: We hypothesize that this regimen of antiprogestin administration may prevent implantation and offer a novel strategy for fertility control. (Am J Obstet Gynecol 1992;167:60-5.)

Key words: RU 486, antiprogestins, contraception, endometrium

It is estimated that the world's population will double by 2050 AD.[1] In a time of limited resources birth control offers the only solution to an otherwise inevitable Malthusian crisis. The United States population has increased by only a modest 10% in the past decade,[1] in part because of a high abortion rate. Many would prefer that population growth be controlled by contraception rather than abortion. However, this goal is compromised by a relatively limited choice or availability of contraceptive technologies. Additionally, there has been a decrease in new research and development initiatives in this field, in spite of the recent emergence of a number of potential approaches. Compounds that block progesterone action represent one such avenue. Progesterone is required for normal reproductive function in women, because it engenders the development of a secretory endometrium and enables the implantation of an embryo.[2] A progesterone antagonist may thus, in theory, impede endometrial development and prevent pregnancy without disrupting other aspects of reproductive function. We previously tested this hypothesis with a large, single midluteal phase dose of RU 486, a 19-norsteroid that antagonizes progesterone action at the receptor level.[3] Although this approach consistently prevented pregnancy in rhesus monkeys,[3] a single late-luteal phase dose was less effective in clinical trials in women.[4] In contrast, our recent studies in guinea pigs demonstrated that daily administration of RU 486 (0.8 to 2.5 mg) throughout an estrous cycle prevented pregnancy in otherwise sexually active, ovulatory animals.[5] Taken together, these results suggested that progesterone blockade of the endometrium would be more effective if initiated earlier in the luteal phase and that a small daily dose of RU 486 might have a cumulative antinidatory effect in women.

We therefore investigated whether RU 486 given at a small daily dose (1 mg) throughout the menstrual cycle could delay endometrial maturation without suppressing steroidogenesis or ovulation in women not at risk for pregnancy. A daily schedule of administration would, in theory, induce a hostile endometrium before the expected time of implantation, regardless of the variability in a given woman's cycle. It would also avoid the difficulty of identifying a specific menstrual cycle day to take the compound. We assessed the develop-

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ment of the endometrium both morphologically, by evaluation of endometrial histology, and functionally, by measurement of serum levels of placental protein 14.[6] Placental protein 14 is synthesized by the progesterone-induced secretory endometrium and is a potent inhibitor of lymphocyte activation, which suggests an important immunomodulatory role during implantation and early pregnancy.[6, 7] Serum placental protein 14 concentrations increase during the late luteal phase and peak at the onset of menses.[6]

Material and methods

Subjects. Eleven healthy nonpregnant women (mean age 31.0 ± 1.5 years, range 23 to 38 years) volunteered for the study. Nine had previously been pregnant and none had a spontaneous abortion. All had regular menstrual cycles of 26 to 30 days and were between 86% and 126% of ideal body weight (mean 64.0 ± 2.3 kg, range 50 to 75 kg). No subject gave a history of galactorrhea, unusual dietary habits, or strenuous exercise, and none had used hormonal contraceptives or intrauterine devices during the previous 6 months. All women used barrier methods or abstained from sexual intercourse during the study. The menstrual cycle before initiation of the protocol was 26 to 30 days in length; ovulation and normal luteal function were confirmed by measuring serum progesterone on three different days of the luteal phase.[8] Pregnancy was excluded by a serum β-human chorionic gonadotropin concentration <2.0 ng/ml (the threshold for detection of pregnancy in this assay) within 48 hours before the onset of RU 486 or placebo administration.

The study was approved by the United States Bureau of Drugs and Biologics and the Clinical Research Subpanel of the National Institute of Child Health and Human Development. Informed consent was obtained from each subject.

Protocol. The study consisted of two treatment cycles separated by one or two rest cycles. Each woman received either RU 486 at a daily oral dose of 1 mg or placebo during one treatment cycle in a randomized, double-blind, crossover fashion. Treatment was started on the first or second day of menses and continued until cycle day 25 or the day of the endometrial biopsy, whichever came last. Hormonal events of the two treatment cycles were monitored by measurements of plasma luteinizing hormone (LH) (daily from day 9 until the midcycle surge) and serum estradiol and progesterone (every 3 days). The endometrium was evaluated by determination of serum placental protein 14 concentrations (every 3 days) and by histologic dating of an endometrial biopsy obtained 7 to 9 days after the LH surge. Electrolytes, liver and renal function tests, and cell blood counts were evaluated at the beginning and end of each treatment cycle. Progesterone was measured on days 18, 21, and 24 of the immediate posttreatment cycle to assess luteal function.[8] Women kept daily records of menstrual flow, premenstrual symptoms, dysmenorrhea, and any unusual symptoms, including intermenstrual bleeding and decreased libido, during the treatment and recovery cycles.

Endometrial biopsy. An endometrial biopsy was scheduled prospectively and obtained 7 to 9 days after the midcycle LH peak was detected by a rapid plasma assay. Specimens were taken from the uterine fundus with the Pipelle curet (Prodimed, Neuilly-en-Thelle, France). Chronologic dating of the biopsies was assigned prospectively by counting forward from the day of the LH peak (taken as cycle day 14) until the day of the biopsy.[9] At the end of the study all biopsies were reviewed independently by two pathologists (M.M. and C.A.), who had no knowledge of the day of the biopsy or the treatment instituted in a given cycle. Histologic dating was done according to the criteria of Noyes et al.[10] with a 2-day reading, e.g., secretory day 22 to 23. Endometrial maturation was assessed by correlating the chronologic date with the most advanced histologic date of the biopsy, in keeping with Noyes et al.'s recommendation that dating should rely primarily on the morphology of the most advanced portion or feature of the endometrium.[10] A biopsy was considered delayed when the histologic dating lagged more than 2 days behind the chronologic dating and normal when these dates were within 2 days of each other.[11]

Preparation of RU 486 and placebo. Capsules containing either 1 mg of micronized RU 486 (Roussel-UCLAF, Paris) or placebo were prepared by the Pharmaceutical Development Service of the National Institutes of Health.

Hormone assays. Measurements of serum estradiol[12] and progesterone[13] and rapid determinations of plasma human chorionic gonadotropin and LH (Diagnostic Products Corporation, Los Angeles) were obtained by radioimmunoassay. The intraassay and interassay coefficients of variation were below 12% and 17%, respectively, for all hormones.

Serum placental protein 14 was measured by radioimmunoassay according to the method of Bolton et al.[14] with minor modifications. The purified placental protein 14 standard (lot no. 120/135) and antiserum (lot no. 201ZA) were kindly provided by Dr. Hans Bohn (Behringwerke AG, Marburg, Germany). Radioiodination of placental protein 14 was carried out by lactoperoxidase, followed by purification of the tracer in Sephadex and concanavalin A-Sepharose columns.[14] The anti–placental protein 14 antibody was used at a dilution of 1:16,000; its specificity has been described elsewhere.[14] Bound and free hormone were separated by adding goat anti-rabbit antiserum and polyethylene glycol. All placental protein 14 samples were measured

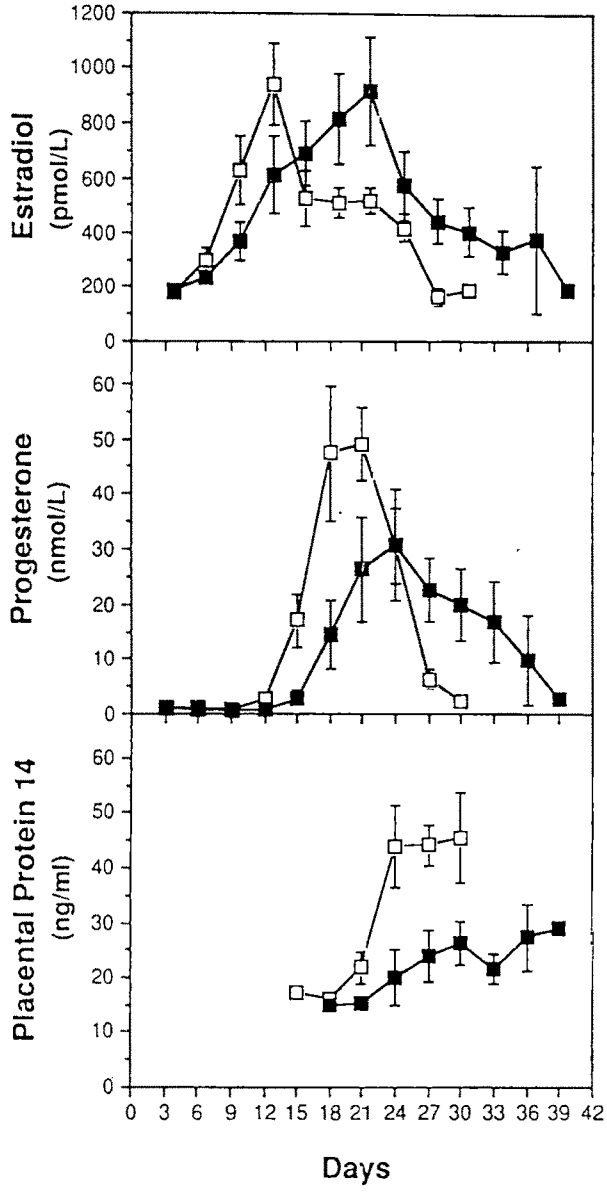


Fig. 1. Effects of RU 486, 1 mg/day *(closed squares)*, and placebo *(open squares)* administration on mean (±SE) levels of estradiol, progesterone, and PP14 in nine ovulatory women. Both medications were given throughout one menstrual cycle. Day 1 corresponds to first day of menses.

in the same assay; the detection limit was 4 ng/ml and the intraassay variation was 6.7%, 4.4%, and 4.4% at concentrations of 31.0, 59.4, and 87.0 ng/ml, respectively.

Analysis. The follicular phase was defined as days elapsed between the onset of menses and the day of the LH peak. The luteal phase was defined as days after the LH peak until the day before the next menses. The integrated secretion of estradiol or progesterone was defined as the sum of all values obtained during a treatment cycle. Results are presented as mean ±SE. Except as noted, the Student t test for paired variables was used to compare results between groups. Differences were considered significant at $p < 0.05$.

Table I. Effects of RU 486 administration (1 mg/day) throughout one menstrual cycle on the duration of the follicular and luteal phases in nine ovulatory women

Treatment	*Cycle length (days)*	*Follicular phase (days)*	*Luteal phase (days)*
Placebo	25.0 ± 0.6	13.0 ± 0.5	12.0 ± 0.6
RU 486	31.4 ± 1.3*	18.9 ± 1.4*	12.6 ± 0.7

*Significantly different compared with placebo ($p < 0.01$).

Results

Effects of RU 486 on cycle length. Two women were excluded from all data analysis. One subject had dyssynchronous endometrial biopsies obtained on the seventh day after the LH peak in both treatment cycles. Because the glandular epithelium and stromal tissue were at different stages of endometrial maturation, it was not possible to assign an overall histologic date to these specimens. In the other woman, the LH surge was suppressed completely during RU 486 administration, and thus no biopsy was obtained. Ovulation probably occurred at least 5 days after RU 486 was discontinued, when progesterone concentrations rose above 3.2 nmol/L. In contrast, her cycle was normal during placebo administration; the LH surge occurred on cycle day 15 and a normal biopsy was obtained on post-LH surge day 8.

RU 486 delayed the midcycle LH surge and prolonged the follicular phase by 1 to 11 days in the remaining nine women (Table I). In contrast, the compound did not change the duration of the luteal phase (Table I). As result, the mean length of the entire cycle increased by an average of 6 days in women receiving RU 486.

Effects of RU 486 on ovarian steroidogenesis. Follicular-phase estradiol concentrations increased at a slower rate and peaked later during RU 486 compared with placebo treatment (Fig. 1). Although not readily apparent from Fig. 1 because of variability of the day on which maximum values occurred, peak preovulatory estradiol levels were significantly higher in the RU 486 (1292 ± 121 pmol/L) than in the placebo group (1098 ± 106 pmol/L, $p = 0.0060$). However, the integrated estradiol secretion was similar in both treatment cycles (4633 ± 705 vs 3495 ± 367 pmol/L/day, respectively). Luteal-phase progesterone concentrations (>3.2 nmol/L) also occurred later during RU 486 treatment and parallelled the delay in the LH surge induced by this compound (Fig. 1). There were no differences in the peak concentration or integrated se-

cretion of progesterone during RU 486 (47.1 ± 8.9 nmol/L and 129.1 ± 20.3 nmol/L/day, respectively) and placebo administration (59.1 ± 10.5 nmol/L and 131.4 ± 10.5 nmol/L/day).

Effects of RU 486 on endometrial maturation. The RU 486 group had a significantly larger difference between histologic and chronologic dating of the biopsy when compared with placebo (Table II). Six of the nine women included in the data analysis had a delayed biopsy during RU 486 treatment, as opposed to none during placebo administration ($p = 0.0027$, χ^2 analysis). In these six women the histologic dating lagged 3 or 4 days behind the chronologic dating of the biopsy. These dates were within 2 days of each other in the remaining three women.

Peak placental protein 14 concentrations (Fig. 1) occurred around the onset of menses and were significantly lower after RU 486 (34.0 ± 3.3 ng/ml) than placebo treatment (49.2 ± 6.5 ng/ml, $p = 0.0103$).

Other effects of RU 486. Premenstrual symptoms, dysmenorrhea, electrolytes, liver and renal function tests, and cell blood counts were unaffected by RU 486 treatment. Likewise, no unusual symptoms, including intermenstrual bleeding and altered libido, were noted.

Both the duration of menses (4.0 ± 0.4 vs 5.9 ± 0.7 days, $p = 0.0492$) and the length of the posttreatment cycle (26.9 ± 0.7 vs 29.0 ± 0.4 days, $p = 0.0026$) were reduced significantly after RU 486 compared with placebo administration. The sum of three luteal progesterone measurements exceeded 47.7 nmol/L in all recovery cycles.

Table II. Effects of RU 486 administration (1 mg/day) throughout one menstrual cycle on endometrial maturation in nine ovulatory women

	*Endometrial maturation**	
Treatment	*Pathologist M.M.*	*Pathologist C.A.*
Placebo	0.2 ± 0.6 (−2 to 3)†	0.3 ± 0.6 (−2 to 3)
RU 486	−2.3 ± 0.6‡ (−4 to 1)	−2.1 ± 0.7‡ (−4 to 1)

*Lag in days between histologic and chronologic dating of an endometrial biopsy, as judged by two different pathologists (M.M. and C.A.).

†Range.

‡Significantly different compared with placebo ($p \leq 0.02$).

Comment

Our data show that administration of the antiprogestin RU 486 at a small daily dose (1 mg) can induce a functional state of progesterone deficiency without suppressing ovarian steroidogenesis or ovulation. Six of the 10 women who ovulated during RU 486 administration had delayed endometrial morphologic characteristics, whereas one exhibited dyssynchrony between glandular and stromal tissue. Although endometrial maturation was assessed only by the histologic criteria of Noyes et al.,[10] it is possible that morphometric analysis, which appears to be more precise,[15] of the endometrium would show abnormalities in the remaining three women who had normal biopsies during RU 486 treatment according to the former method. The suppressed serum concentrations of placental protein 14, a glycoprotein synthesized by the progesterone-induced secretory endometrium that has been proposed as a marker of endometrial function,[6] provides additional evidence for the antiprogestin effects of RU 486 on the endometrium. The pharmacologically induced abnormalities in endometrial morphology and function observed in this study are similar to those seen in infertile women with luteal-phase defects. This condition, most often due to deficient progesterone secretion, is typically associated with a delayed endometrial biopsy.[11] Although placental protein 14 concentrations have not been reported in women with luteal-phase defects, an immunologically identical protein, progestagen-dependent endometrial protein,[6] was shown to be reduced in this population.[16] Thus the endometrial abnormalities observed in the present study may reflect an intrauterine environment hostile to implantation.

The time course of endometrial maturation during RU 486 administration deserves further investigation. The findings of the Carnegie Institute that both the embryo and the endometrium are at luteal phase day 6 to 8 at the beginning of implantation in cycles resulting in conception suggest that successful initiation of this process requires a relative synchrony between the development of the embryo and the maturation of the endometrium.[17] These results influenced our decision to examine the endometrium at the expected time of implantation relative to the LH surge and embryo development rather than at a later time in the luteal phase, as is often done in clinical practice.[11] Also, histologic dating of the endometrium is more precise and agrees more closely with chronologic dating when specimens are obtained in the midluteal than in the late luteal phase.[18, 19] It is possible, however, that a delayed endometrium 7 to 9 days after the LH surge might later mature and sustain implantation, if the blastocyst could wait until maturation occurred. Studies of women with ovarian failure undergoing assynchronous transfer of donor embryos suggest that implantation can occur if the endometrium is no more than 2 days delayed, as assessed by an endometrial biopsy performed in a previous cycle.[20] Because the endometrium in these recipients is prepared with exogenous steroids and the embryo develops in vitro until transferred, it is unclear whether these findings can be extrapolated to the blas-

tocyst-uterine relationship in natural cycles resulting in conception.

Although RU 486 administration delayed the LH surge and ovulation by an average of 6 days, the length of the follicular phase and the total duration of the RU 486 cycle remained within the normal range for the adult female population.[21] The prolongation of the follicular phase may reflect partial suppression of folliculogenesis induced by RU 486, as suggested by the slower rise in follicular-phase estradiol concentrations. This may be mediated through either gonadotropin inhibition, a direct ovarian effect, or a combination of both.[22, 23] However, the significantly higher preovulatory estradiol levels observed during RU 486 administration suggest that another late follicular phase mechanism contributes to the delay in the gonadotropin surge. Because progesterone concentrations start to rise just before the LH surge and may trigger this process, progesterone antagonism induced by RU 486 at midcycle may delay this event.[24]

RU 486 administration did not change the duration of the luteal phase. Both RU 486 and placebo cycles had a 12-day luteal phase, which is slightly shorter than usually seen in normally cycling women.[25] This may be due to the luteolytic effect of prostaglandins released during the endometrial biopsy or to partial disruption of the endometrium caused by this procedure.[10]

Although follicular-phase estradiol concentrations increased at a slower rate during RU 486 treatment, the integrated estradiol secretion was similar to that seen during placebo administration, indicating that the RU 486 regimen did not cause a low estrogenic state. Likewise, RU 486 did not alter corpus luteum function, as evidenced by similar peak levels and integrated secretion of progesterone in both treatment cycles. Although the blood sampling frequency of every 3 days has been advocated as a good index of luteal function,[8] this approach may not detect subtle changes over the entire cycle or in an event that occurs over a short time span, such as the preovulatory peak of estradiol concentrations.

RU 486 can thus preserve ovulation, steroidogenesis, and the timing of the menstrual cycle when given at a daily dose of 1 mg. This contrasts with the complete inhibition of follicular development, steroidogenesis, and ovulation and also with the luteolysis seen with higher doses.[3, 22, 23] Ovulation was suppressed in our preliminary clinical trials even at a daily dose of 10 mg (unpublished data). The dose of 1 mg used in the current study did not consistently preserve ovulation while inhibiting endometrial development, as one woman was anovulatory and three had normal endometrial histology. These results suggest that this dose was too high in the first and too low in the latter cases. These differences in dose-response may reflect individual variations in absorption or metabolism of RU 486 and indicate that further work, including serum measurements of this compound, will be needed to define an optimal dose of the drug for contraceptive trials.

No adverse effects were observed during RU 486 treatment, suggesting that it can be safely administered in the regimen used in the current study. The subsequent menses were shortened, however, most likely due to RU 486 inhibition of endometrial development. The post–RU 486 cycle was ovulatory but slightly shortened. The mechanism by which this occurred was not apparent from the available data.

In summary, at a daily dose of 1 mg, RU 486 disrupted both the morphology and function of the endometrium while preserving steroidogenesis, ovulation, and timing of the cycle. Taken with our previous findings that daily administration of RU 486 prevents pregnancy in the guinea pig,[5] it is tempting to speculate that daily administration of an antinidatory dose of a progesterone antagonist may offer a novel strategy for fertility control. Although the dose used in women (0.016 mg/kg/day) was proportionally lower than in guinea pigs (1 to 3 mg/kg/day), this comparison may not be valid because of interspecies differences. Because our study group was small, the present data must be regarded as preliminary. However, this study demonstrates that, in principle, a small daily dose of an antiprogestational agent can inhibit both morphologic and functional maturation of the endometrium and thus provides a rationale for further investigations to elucidate the contraceptive potential of these agents in women.

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Antenatal tests of fetal welfare and development at age 2 years

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OBJECTIVE: Our objective was to examine the outcomes at 2 years of age of fetuses delivered electively before 34 weeks, studied antenatally with two tests of fetal well-being.

STUDY DESIGN: Forty-two fetuses from high-risk pregnancies delivered electively by cesarean section before 34 weeks were stratified into normal versus abnormal subgroups with umbilical Doppler flow velocity waveform and fetal heart rate results. Developmental outcome was assessed at 2 years. Two comparison groups were also selected: 40 matched premature controls delivered spontaneously before 34 weeks and 67 normal babies delivered spontaneously at term. Frequency outcome data were tested with χ^2 analyses and the remainder with analyses of variance.

RESULTS: Within the electively delivered study group poor cognitive progress at 2 years was more strongly associated with an abnormal fetal heart rate result than an abnormal Doppler result. Compared with the premature control and normal term groups, electively delivered fetuses were significantly delayed in growth, cognition, and motor development ($p < 0.005$).

CONCLUSIONS: Adverse fetal welfare in a high-risk obstetric sample was associated with poorer outcome at 2 years. However, the whole of the group of fetuses from such high-risk pregnancies showed significant developmental delay compared with normal term children and, more importantly, matched premature infants delivered spontaneously from otherwise uncomplicated pregnancies. (Am J Obstet Gynecol 1992;167:66-71.)

Key words: Fetal compromise, high-risk pregnancy, Doppler, fetal heart rate, early childhood development

It is a goal of obstetrics to ensure that a fetus is born well and, through growth and development after birth, able to attain its full biologic potential. Antenatal tests of fetal well-being exist as aids to the obstetrician to determine the appropriate time for intervention and delivery. Clinical suspicion of fetal compromise is usually followed by ultrasonographic assessment of size and biophysical profile. Nonstressed fetal heart rate (FHR) monitoring has become the most widely used test of fetal condition and need for delivery. A normal tracing encourages the obstetrician to maintain the fetus in utero to gain maximal maturity. A persistent nonreactive tracing indicates encroachment on critical fetal reserve and the time for intervention. More recently, Doppler studies of the umbilical artery flow velocity waveform have been introduced.[1] Such studies identify a vascular pathologic condition in the fetal placenta.[2] Their use is based on the premise that this lesion precedes fetal effect and identifies pregnancies for intensive fetal surveillance.

There is increasing awareness that long-term developmental handicaps may originate from fetal insult during the antenatal period rather than during labor.[3, 4] Many descriptive studies have examined the relationship between tests of fetal well-being and condition at birth. However, there has been very little information relating fetal welfare test results with later infant and child development. In the current study we have investigated development at 2 years of age in a group of fetuses born after complicated pregnancies in whom Doppler umbilical flow studies and nonstressed FHR monitoring were conducted.

Patients and methods

Patients. The study group comprised children who had been delivered electively (by cesarean section) from high-risk pregnancies before or at 34 weeks' gestation. All had been assessed with Doppler flow velocity waveform studies within 2 weeks of delivery and FHR mon-

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itoring within 3 days of delivery. They were all delivered at Westmead Hospital between 1986 and 1988 and had been identified for follow-up during pregnancy. This study was approved by the Research and Ethics Committee of Westmead Hospital.

At the 2-year follow-up 78 eligible subjects were contacted by mail and invited to participate. Forty-two subjects agreed to participate, 14 refused, and no contact was made with 22 subjects. There were no significant differences between participants and nonparticipants in gestation, sex, birth weight, antenatal test results, maternal age, or hospital health insurance status (used here as a broad indicator of social class) ($p > 0.05$). Clinical details of the 42 subjects in the study group are displayed in Table I. Gestation was determined by patient dates and, in 31 cases, confirmed by an ultrasonographic scan before 22 weeks. The major reasons for elective delivery were suspected or known fetal compromise ($n = 26$) and maternal hypertension ($n = 15$). In one case antepartum hemorrhage associated with placenta previa was the reason.

The antenatal results of the placental Doppler umbilical flow velocity waveform studies and FHR monitoring were used to divide the study group into normal and abnormal groups. The Doppler umbilical artery flow velocity waveforms were recorded as previously reported.[5] The systolic diastolic ratio was used as an index of downstream resistance. This result was classed as abnormal if it was above our 99.95th percentile.[6] This division was chosen as the statistical cut-off equivalent to absent diastolic flow in fetuses of 28 to 32 weeks' gestation. The nonstressed FHR trace was recorded at a paper speed of 3 cm/min. The abnormal FHR group included those fetuses with a nonreactive trace, defined by an FHR baseline variability <5 beats/min and no accelerations with or without late decelerations in response to Braxton Hicks contractions.[7] This stratification of the 42 children resulted in 17 normal and 25 abnormal Doppler study cases and 26 normal and 16 abnormal FHR cases. The correlation between Doppler study and FHR result was $r = 0.25$ ($p = 0.057$).

For comparison two further groups were selected for study. A control group delivering before 34 weeks after spontaneous onset of labor was chosen. This group ($n = 40$) was matched to the electively delivered study group for gestation (±1 week) and health insurance status. All children had birth weights >5th percentile and Apgar scores at 1 and 5 minutes of ≥5 and 7, respectively. A group of fetuses delivered spontaneously at term (37 to 42 weeks' gestation) was also selected to provide a normative range. In this group (67 patients) all birth weights were >5th percentile, and Apgar scores at 1 and 5 minutes were ≥7 and 9, respectively. Complete antenatal testing was not available for the premature control and term groups, but case selection ensured there was no history of obstetric complications or evidence of fetal compromise during pregnancy.

Table I. Clinical details of electively delivered pre-34-week study group

Cases (No.)	42
Gestation (wk, mean ± SD)	30.8 ± 2.0
Birth weight (gm)	
Mean	1163
Range	580-2300
Boys (No.)	14 (33%)
Mother's age (yr, mean ± SD)	26.4 ± 5.5
Nulliparous (%)	57
Private health insurance (%)	60

Methods. Children were reviewed at 2 years of age, corrected for gestation, and assessed for physical, cognitive, and language outcome. Mean corrected age at time of assessment was 24.9 months, with a range of 21 to 30 months.

Weight, height, and head circumference measurements were recorded and percentiles calculated.[8, 9]

The Denver Developmental Screening Test[10] was administered. This test is designed to screen children for risk of delay in personal-social, fine motor, gross motor, and language development. The scoring of this test was modified so that the number of failures and the areas in which they occurred were recorded.

The Bayley Scales of Infant Development[11] were used to assess the achievement of milestones at 2 years. The test provides a Mental Development Index incorporating fine motor skills, eye-hand coordination, and some language items, and a Psychomotor Development Index examining coordination and balance of the larger leg muscles.

Language progress was tested with the Sequenced Inventory of Communication Development.[12] The test comprises two scores: a receptive score relating to the child's understanding of language and an expressive score reflecting the child's ability to produce sounds and words.

In addition, parents supplied demographic details such as level of schooling and occupation to index social class.[13] Information on the home environment was obtained with three subtests from Caldwell's HOME scale,[14] labeled maternal responsivity, maternal involvement, and provision of toys and play materials. These subtests were selected because of the strong correlations reported with early development.[15, 16]

Statistics. Data were corrected for gestation where appropriate. In all comparisons the eight outcome mea-

Table II. Outcome at 2 years of age in electively delivered study group stratified by Doppler and FHR results

Assessment	Doppler		FHR	
	Normal (n = 17)	*Abnormal (n = 25)*	*Normal (n = 26)*	*Abnormal (n = 16)*
Physical				
Weight (kg)	11.7	11.2	11.5	11.1
Height (cm)	85.0	83.3	84.2	83.7
Head circumference (cm)	48.6	47.5 (0.08)	47.9	47.9
Denver Developmental Screening Test (% failed ≥1 item)	18	16	8	31 (0.09)
Bayley				
Mental Development Index	96	96	100	87 (0.05)
Psychomotor Development Index	93	90	94	85 (0.07)
Sequenced Inventory of Communication Development				
Receptive score (% >1 SD below mean)	35	16	19	31
Expressive score (% >1 SD below mean)	41	28	27	44

Numbers in parentheses represent p values (one-tailed) <0.10.

sures at 2 years corrected age were weight; height; head circumference; number of items failed on the Denver Developmental Screening Test, Bayley Mental Development Index, and Psychomotor Development Index, and receptive and expressive Sequenced Inventory of Communication Development language scores. Frequency data (Denver Developmental Screening Test score, Sequenced Inventory of Communication Development scores) were tested with χ^2 analyses. The remaining data (growth parameters, Bayley Mental Development Index and Psychomotor Development Index) were analyzed with analyses of variance, which included sex, social class, and the HOME total score as covariates. It is well established that these three factors relate to child development, and using covariate analysis was intended to increase the power of the statistical test by reducing the error variance.

Data were tested in two stages. To examine the value of the two antenatal tests the outcome data within the electively delivered study group were analyzed comparing the normal and abnormal groups for each fetal test. The effect of the other antenatal test result was controlled in the analyses of variance with covariate analysis. This ensured that any significant differences were not confounded by the other antenatal test result.

Comparisons were then made between the elective study group and the premature control and the normal term infants. The developmental status of the study group relative to the control groups was also analyzed with both antenatal test divisions. A p value of 0.005 was considered significant to minimize type I errors. Significant differences were tested with post hoc, pairwise χ^2 tests or Tukey tests on adjusted means, with a significance level of $p < 0.01$ to correct for multiple comparisons.

Results

Differences within the study group. Outcome data within the electively delivered group stratified by Doppler and FHR results are displayed in Table II. Stratification by the Doppler umbilical fetal velocity waveform result revealed no significant differences ($p > 0.05$). Comparison of the FHR results showed a significant difference on the Bayley Mental Development Index ($p < 0.05$). Trends were apparent on the Bayley Psychomotor Development Index ($p = 0.07$) and Denver Developmental Screening Test ($p = 0.09$). An analysis of the subgroup in whom both the Doppler and FHR results were abnormal showed trends similar to the abnormal FHR group, particularly on the Bayley tests (Mental Development Index $p = 0.13$, Psychomotor Development Index $p = 0.04$).

Although the purpose of the study was the investigation of antenatal well-being and outcome at 2 years we also examined several neonatal measures from the newborn period, for possible confounding effects. Comparisons of the study group stratified by either Doppler or FHR result revealed no significant differences between the normal versus abnormal groups on the following variables: total days in neonatal intensive care, total time on oxygen, incidence of necrotizing entercolitis, or incidence of cerebroventricular hemorrhage.

Differences between the study group and control groups. Table III displays the outcome data of the electively delivered study group and the two control

Table III. Outcome at 2 years of age in electively delivered study group and in premature control and normal term groups

Assessment	*Elective group (n = 42)*	*Premature control group (n = 40)*	*Normal term group (n = 67)*	*Result*
Physical				
Weight (kg)	11.5	12.2	13.0	$p < 0.005$*
Height (cm)	84.3	85.0	87.1	$p < 0.005$*
Head circumference (cm)	48.2	48.8	48.9	–
Denver Developmental Screening Test (% failed ≥1 item)	17	5	3	
Bayley				
Mental Development Index	96	107	111	$p < 0.005$*
Psychomotor Development Index	91	102	105	$p < 0.005$*
Sequenced Inventory of Communication Development				
Receptive score (% >1 SD below mean)	24	13	3	–
Expressive score (% >1 SD below mean)	33	8	10	–

*Analysis of variance.

groups. Post hoc analyses showed the electively delivered study children were significantly smaller than term babies in both weight and height ($p < 0.01$). Babies in the premature control group were also shorter than term babies ($p < 0.01$). On both Bayley indexes the study group scored significantly below both control groups ($p < 0.01$).

The study group was divided by the results of the umbilical Doppler and FHR tests and compared with the control groups, with the data displayed in Tables II and III. Analyses indicated the electively delivered study children in whom the Doppler results were abnormal performed significantly below the normal term group in all growth parameters and both Bayley indexes ($p < 0.01$). The abnormal Doppler group also differed from the premature control group on the Bayley Psychomotor Development Index ($p < 0.01$). In the subgroup with a normal Doppler result developmental scores were consistently lower, but none of these differences were significant.

Using the FHR test division both the abnormal and normal study children had lower weight and height at 2 years compared with children delivering at term ($p < 0.01$). The abnormal FHR group scored significantly below both control groups on the Bayley indexes ($p < 0.01$). More children in the abnormal FHR group failed at least one item (31%) on the Denver Developmental Screening Test compared with both the premature (5%) and the term (3%) control groups ($p < 0.01$). Analysis of variance results of the receptive language scores on the Sequenced Inventory of Communication Development were significant ($p < 0.005$), but post hoc tests did not detect a pairwise difference at $p < 0.01$. Differences existed between the normal FHR study group and controls on the Denver Developmental Screening Test and Bayley tests but did not reach significance.

Comment

This study was designed to evaluate growth and development of children at 2 years of age against the results of fetal welfare tests carried out during pregnancy in a high-risk obstetric population. It was expected that children with abnormal fetal welfare test results would fare worse than those with normal results. We found that fetuses delivered electively before 34 weeks did show some differences at 2 years when divided by the result of antenatal tests of fetal well-being. The subgroup with an abnormal result was developmentally behind the normal subgroup in many areas, but few differences reached statistical significance. We found the result of FHR monitoring rather than umbilical Doppler study was a better discriminator of developmental progress at 2 years. Umbilical Doppler studies are an early detector of placental vascular pathologic conditions, which are believed to lead to fetal constraint. An abnormal result in this test will include cases in which the fetus has been affected and others in which this has not yet occurred. It is therefore not surprising that the difference between the normal and abnormal Doppler subgroups is small. In contrast, an abnormal FHR result indicates that a fetal effect has

occurred. The differences found between the normal and abnormal FHR subgroups support this point.

On the basis of results within our study group showing only a small difference in outcome for infants with an abnormal versus normal antenatal test, it could be concluded that, in spite of an adverse antenatal environment, timely delivery aided by the results of fetal welfare tests has allowed the fetus to emerge relatively unscathed. However, we have shown that, as a group, children from complicated pregnancies delivered electively before 34 weeks performed markedly below peers selected from uncomplicated normal term pregnancies. This is consistent with previous studies.[17, 18] More importantly, the electively delivered children also exhibited delays compared with a matched preterm group who had delivered spontaneously before 34 weeks. The children delivered electively from high-risk pregnancies showed poorer performance at 2 years in physical growth, cognition, and motor performance when compared to both control groups. Further analyses indicated that in particular children with an abnormal antenatal test result, whether Doppler or FHR, were indeed exhibiting the most developmental deficits. However, the study children in whom the fetal welfare test results were normal were also consistently behind their peers in the control groups, although not always reaching statistical criteria. Although the number of cases in the subgroups are small, the differences between the elective study group as a whole and the control groups are highly significant.

In this study we assessed only 54% of the electively delivered infants identified for follow-up from the antenatal period. We have compared participants and nonparticipants on gestation, sex, birth weight, antenatal test results, maternal age, and hospital health insurance status and found no differences. The possibility of bias, however, is not excluded. Among our control groups we do not have details of Doppler and FHR results. These pregnancies were uncomplicated, and there was no reason for these tests to be performed. Our control groups may have included some cases of unrecognized fetal compromise, but the effect of this would have been to reduce the differences between the groups. Our findings may in fact underestimate the true disparity.

The poor overall performance of our study group at 2 years raises questions about the timing of fetal injury and the feasibility of preventing it by early intervention (delivery). There is no doubt nonstressed FHR monitoring facilitates delivery of a fetus in the best possible condition at birth. This short-term role should not be undervalued. Our data would question the long-term benefits of this course of management in ensuring normal developmental outcome. Clearly there exists scope for the pursuit of other avenues of fetal surveillance and management if obstetric intervention is to be timed to precede fetal damage. It may be that fetal injury is a cumulative result of adverse circumstances operating since early in the pregnancy and therefore not totally preventable by current obstetric practice. It is likely the same considerations are relevant when considering major handicaps, such as cerebral palsy, originating from antenatal insult.

In addition to the effects of fetal well-being the importance of the early social and emotional environment of the child must be considered. Perinatal complications have been found to be associated with poorer physical and psychologic development only when combined with a persistently poor environment.[18-20] Given the potential palliative role of the child's early postnatal environment, it appears that evaluation of targeted remedial parenting programs for infants delivered electively before term is also warranted. This represents a new and important approach to therapy.

In summary, we have shown in this study that in the presence of potential fetal compromise delivery before the development of a nonreactive FHR tracing is associated with a better outcome in terms of growth and development at the age of 2 years. However, it does appear that such pregnancies yield fetuses who, as a group, exhibit suboptimal early childhood development. By the time the signs of fetal compromise are present fetal damage has already occurred. This points to the need for new approaches in management.

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Retrospective maternal mortality case ascertainment in West Virgina, 1985 to 1989

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OBJECTIVE: The death of women from pregnancy-related causes remains a threat to national maternal and child health. Maternal deaths as persistent, albeit rare occurrences are overlooked if vital registration systems are relied on to report such deaths.

STUDY DESIGN: Live birth records were matched with death records for women of reproductive age to detect if a woman died within 1 year of delivery. The data for potential cases were reviewed by committee and classified as maternal and nonmaternal deaths.

RESULTS: Of all linked birth-death records, 32% were related to pregnancy: 81% were directly related to pregnancy and 19% were indirectly related to pregnancy. The most frequent causes of death were hemorrhage and embolism. Thirty-eight percent of the women were transferred to tertiary hospitals before death. The case ascertainment through this study improved maternal death detection by 100% over official vital statistics.

CONCLUSION: Enhanced maternal mortality surveillance increased the detection of maternal death in West Virginia. Case review of these deaths yielded important information useful in shaping the state's perinatal system. (AM J OBSTET GYNECOL 1992;167:72-6.)

Key words: Maternal mortality, vital registration, mortality review, record linkage, West Virginia

The death of women from causes related directly or indirectly to pregnancy and childbearing remains a persistent threat to maternal and child health not only in developing countries[1] but also in the United States.[2] The existence of state and locally based maternal mortality review committees (e.g., North Carolina,[3, 4] Iowa,[5] Oklahoma,[6] and Massachusetts[7]) have enabled long-term analysis of trends in maternal death. In addition, the establishment of the Maternal Mortality Collaborative of the American College of Obstetricians and Gynecologists has enabled improved surveillance of maternal mortality in selected areas of the United States.[2] In states where maternal mortality review committees do not currently exist, for example, West Virginia, the existence of maternal death as an important and persistent, albeit relatively rare, occurrence may be overlooked if traditional vital registration systems are relied on as the only sources of reporting such deaths. Death certificate information available through traditional vital registration systems are not adequate for enhanced detection of maternal deaths, the American College of Obstetricians and Gynecologists Maternal Mortality Collaborative found that maternal death was underreported by vital registration in selected areas of the United States by 39% during 1980 through 1985.[2] In particular, in West Virginia, where the provision and organization of obstetric and medical care has reached crisis proportions in recent years,[8] enhanced maternal mortality surveillance is an important activity in assessing the current status of maternal and child health.

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Material and methods

Record linkage and data sources. According to the methods of other similar studies of maternal death,[9, 10] we matched all deaths among West Virginia resident women aged 10 to 49 years (n = 2623) and out-of-state resident women dying in West Virginia (n = 351) between 1985 and 1989 against all live births to West Virginia residents (n = 138,178) and out-of-state residents (n = 17,454) between 1984 and 1989. Seven maternal deaths were reported through the state's vital registration system for this period; all seven deaths were also identified through the linkage procedure. The woman's death certificate was matched with the baby's birth certificate if the woman died within 1 year of the birth. The record match was based on social

security number, mother and baby's name, and mother's date of birth. All matches were hand-verified to assure correct linking of the mother-infant pair. This record linkage yielded a cohort of women dying within 1 year after giving birth ($n = 49$). Additionally, all women with causes of death from *International Classification of Diseases, Ninth Revision* Codes 630 through 679 (Complications of Pregnancy, Childbirth, and Puerperium), who were not identified by the record linkage ($n = 1$) were added to the linked records to be reviewed. Because of a lack of unique case-identifying information, record matches with other pregnancy outcome records (i.e., abortion records, fetal death records) were not possible for the time of this study. The final set of matched-identified potential maternal death cases ($n = 50$) represents all women dying within 1 year after giving birth, including women previously identified through vital registration as dying of maternal causes regardless of termination status (e.g., ectopic pregnancy). Data assembled for all potential maternal death cases ($n = 50$) included the woman's death certificate and baby's birth certificate (certificate not available for one ruptured tubal pregnancy). Medical records for the woman and the baby, including autopsy reports, were requested for all women dying of non-injury-related causes ($n = 28$). Hospital and medical records were obtained for the woman ($n = 27$) and the baby ($n = 24$), and autopsy reports were obtained for 12 women (of 15 autopsies conducted).

Obstetric review. The data assembled for all potential maternal death cases ($n = 50$) were reviewed by two in-state and two out-of-state obstetricians. The protocol was approved by the West Virginia University Institutional Review Board.

The purpose of the maternal death review team was to categorize each case as a maternal or nonmaternal death. Maternal deaths were further defined as direct maternal deaths or indirect maternal deaths. Operational definitions of maternal death, direct maternal death, indirect maternal death, and nonmaternal death, on the basis of definitions used by the Maternal Mortality Collaborative[2] and the Massachusetts Medical Society's Committee on Maternal Welfare,[11] as applied in this study were as follows:

Maternal death. A maternal death is a death caused or contributed to by pregnancy occurring during pregnancy or within 1 year after the end of pregnancy.

Direct maternal death. A direct maternal death is a death resulting from complications of pregnancy, labor, delivery, or their management. Also, a maternal death is one resulting from intervention elected or required by the pregnancy or resulting from the chain of events initiated or complicated by the intervention.

Indirect maternal death. An indirect maternal death is a death in which pregnancy exacerbated a preexisting health problem. Also, an indirect maternal death results from a disease developing before or during pregnancy that was aggravated by the physiologic effects of the pregnancy and caused death.

Nonmaternal death. A nonmaternal death is a death occurring during pregnancy or within 1 year of termination of pregnancy but considered unrelated to pregnancy, its complications, or management (e.g., accident, homicide, suicide).

The final case designations (direct maternal death, indirect maternal death, and nonmaternal death) were reached by consensus among the obstetric reviewers.

Analysis. To quantify the magnitude of maternal death in West Virginia, crude maternal mortality ratios are presented for each year of the study period (1985 through 1989). Maternal mortality ratios were calculated as the number of maternal deaths during a specified period ($\times 100{,}000$) divided by the total number of live births during the same period.[12] The resulting ratio reflects the number of women dying of maternal causes per 100,000 live births. Because mode of delivery information was collected only for 1989, maternal mortality rates specific to vaginal and cesarean deliveries could not be computed. To describe the circumstances and characteristics of maternal death in West Virginia, basic frequency distributions are presented for demographic characteristics and delivery characteristics of all maternal deaths.

Results

Forty-nine women dying within 1 year after giving birth were identified by the record linkage procedures and were subsequently reviewed as potential maternal deaths (Fig. 1). In addition, one woman whose death resulted from a ruptured ectopic pregnancy that did not terminate in a live birth was identified through vital registration coding.

Of these 50 deaths reviewed, 16 (32%) were determined to be directly or indirectly related to the woman's pregnancy, its management, or complications. Thirteen of these maternal deaths (81%) were determined to be directly related to the pregnancy, whereas three deaths (19%) were indirectly related to the pregnancy. Thirty-three cases (66%) were determined unrelated to the woman's pregnancy: 22 cases (67%) were attributable to injuries (including accidents, homicide, and suicide) and 11 cases (33%) were attributable to non-injury-related causes. Insufficient information was available for one case; therefore a definitive designation could not be assigned.

Overall, the maternal mortality ratio for the 1985 through 1989 study period, or the risk of death from maternal causes among women giving birth during this

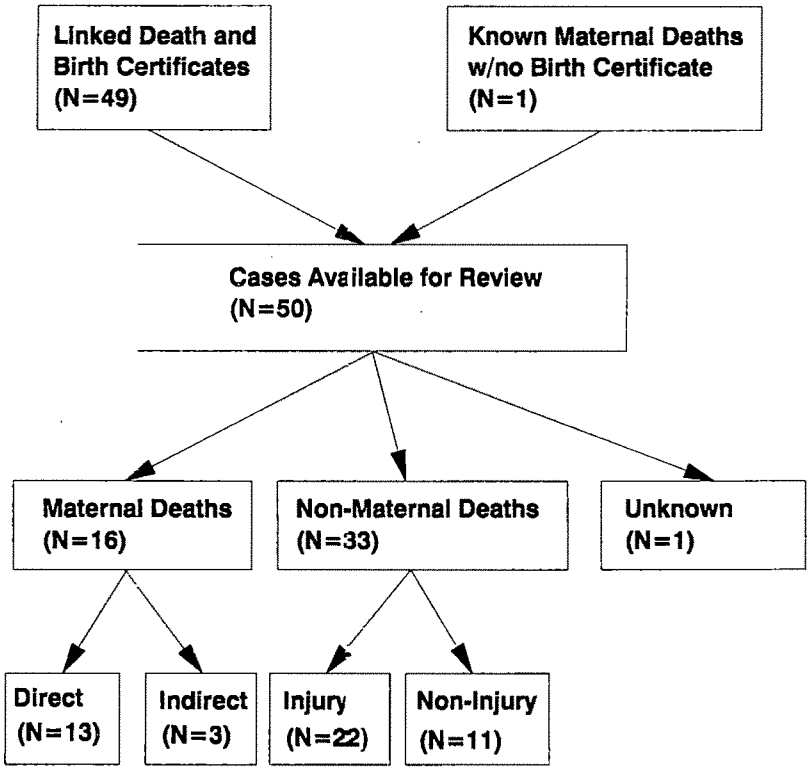


Fig. 1. Disposition of potential cases, maternal mortality study, West Virginia, 1985 through 1989.

period, was 12.4 maternal deaths per 100,000 live births (Fig. 2). The ratio increased from 3.8 and 3.9 in 1986 and 1987 (respectively) to 12.1 in 1988 and 31.9 in 1989. The actual number of maternal deaths identified between 1985 and 1988 (eight deaths) equaled the number of deaths identified from 1989 alone.

All women dying of maternally related causes between 1985 and 1989 were <40 years old (Table I), with one death (6%) in a woman <20 years old, 10 deaths (63%) in women between 20 and 29, and five deaths (31%) in women between 30 and 39. Fourteen white women (88%) and two black women (13%) died of maternal causes during the study period. Eleven maternal deaths (69%) were in women residing in rural, non–Standard Metropolitan Statistical Areas. As shown in Table I risk of maternal death was higher among black women and among older (≥30) women.

All women died within 90 days of giving birth: 10 women (63%) died within 7 days of delivery, three additional women (19%) died between 8 and 30 days of delivery, one woman (6%) died between 31 and 42 days of delivery, and two women (13%) died between 43 and 90 days of delivery. Nine women (56%) gave birth in non–level III hospitals, and seven women (44%) died in non–level III hospitals. Six women (38%) were transferred to level III hospitals either before (two women) or after delivery (four women). The primary mode of delivery for women dying of maternal causes in this study was cesarean section (69%), with seven emergency and four planned cesarean sections indicated in the records. Half of the women (50%) were delivered at term.

The most frequent immediate causes of maternal death were hemorrhage (25%) and embolism (19%). Two women each died of cardiomyopathy and hypertensive disease, and one woman each died of infection and complications of anesthesia. Three women (19%) died of other indirect causes. In addition, four maternal deaths (25%) were complicated by preeclampsia, four deaths (25%) were complicated by respiratory problems, and three deaths were complicated by anesthesia. Motor vehicle accidents contributed to 15 nonmaternal deaths (45%), with cancer contributing to five nonmaternal deaths (15%), suicide contributing to four nonmaternal deaths (12%), and homicide contributing to three nonmaternal deaths (9%).

Finally, the woman's recent pregnancy status was mentioned in the medical records of all 16 maternal deaths. The death certificates of 11 maternal deaths mentioned or referred to the woman's recent pregnancy, and seven maternal deaths were coded and reported previously from the death certificates as maternal deaths.

Comment

The magnitude of maternal mortality in West Virginia during the period of 1985 to 1989 is difficult to

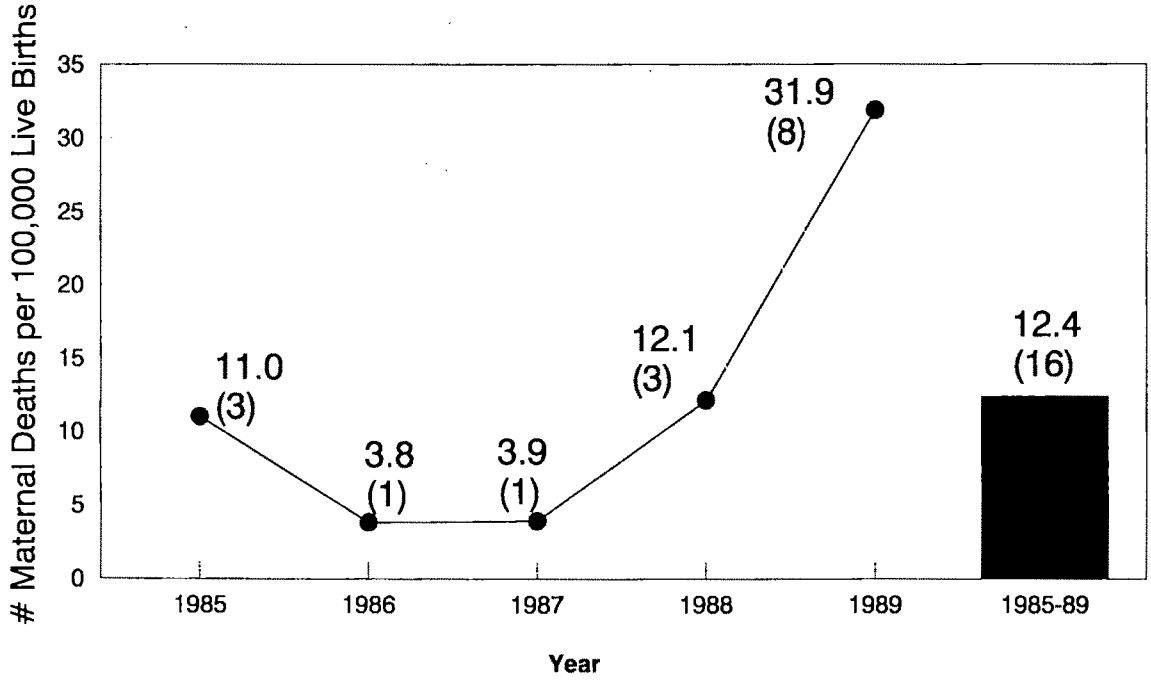


Fig. 2. Maternal mortality rates, West Virginia, 1985 through 1989.

Table I. Demographic characteristics of maternal deaths, West Virginia, 1985 through 1989

Characteristic	*Maternal deaths*		*Live births*		*Rate (per 100,000 births)*
	No.	*%*	*No.*	*%*	
Total cases	16	100	128,932	100	12.4
State of residence					
West Virginia	13	81	113,627	88	11.4
Other	3	19	15,305	12	19.6
Age at death (yr)					
<20	1	6	21,556	17	4.6
20-29	10	63	80,302	62	12.5
30-39	5	31	27,036	21	18.5
Race					
White	14	88	123,315	96	11.4
Black	2	13	4,981	4	40.2
Area of residence					
Standard metropolitan statistical areas	5	31	37,409	29	13.4
Non–standard metropolitan statistical areas	11	69	91,523	71	12.0

interpret in a national context; annual maternal mortality ratios published by the National Center for Health Statistics are based on vital registration information alone, which has been shown to underreport maternal death. The national maternal mortality ratio of 8.0 maternal deaths per 100,000 live births for 1988, the highest national ratio since 1983,[13] while most likely underreporting true maternal mortality still indicates a slight increase in maternal mortality over 1987 (6.1 maternal deaths per 100,000 live births). Importantly, West Virginia's maternal mortality ratio increased in the last 2 years (1988 and 1989) of the study period, essentially reflecting this increaase in maternal mortality nationwide. Furthermore, substantial effort must be devoted to reducing maternal mortality in West Virginia in concordance with the year 2000 goal of no more than 3.3 maternal deaths per 100,000 live births.[14]

The major causes of maternal death in West Virginia (hemorrhage, embolism, hypertensive disease) reflect national findings.[2] This study, however, most likely underestimates true maternal mortality in West Virginia because the method used will not detect deaths attributed to maternal causes, either directly or indirectly, unless (1) the pregnancy resulted in a live birth or (2)

pregnancy status was noted on the woman's death certificate and the certificate was coded and reported as a maternal death. Information collected nationally indicates that approximately 52% of all maternal deaths are to women whose pregnancy resulted in a live birth.[15] Additionally, some nonmaternal deaths (e.g., suicide, accidents) may indeed be maternally related although not through the readily identifiable medical information available to reviewers. For these reasons the findings of this study may underreport true maternal mortality in West Virginia if such mortality includes deaths to women whose pregnancies did not terminate with a live birth or if the cause of death appeared to be nonmaternal (e.g., suicide).

The review committee approach to identifying maternal deaths for the study period enhanced the state's detection of reproduction-related deaths. Although vague or absent legal protection of findings from such review groups and, indeed, participation on such review groups threatens maternal mortality review throughout the United States,[16] West Virginia's *Code* explicitly provides immunity from liability for the review group and protects the group's findings from being subject to subpoena or discovery.[17] Importantly, the case ascertainment achieved through this study design improved maternal death detection in West Virginia by 100% over traditional vital statistics mechanisms. The woman's pregnancy status was mentioned in the medical records of all maternal deaths; however, only seven maternal deaths were officially reported by vital statistics. The failure to detect five of these maternal deaths was due to pregnancy status being missing from the death certificate. An additional four maternal deaths with pregnancy status indicated on the death certificate were not coded and reported as maternal deaths. Clearly, improvements in the recording and coding of maternal cause of death could improve traditional vital statistics systems capabilities substantially in the detection of maternal death.

We thank the medical records departments of all involved hospitals and the Office of Chief Medical Examiner for assistance and cooperation in releasing medical records information and autopsy reports. We also thank Joe Kennedy, Tom Leonard, Gary Thompson, and Tom Light of the West Virginia Health Statistics Center for their technical assistance in conducting the computer linkages for this research and Jack Smith and Lisa Koonin from the Division of Reproductive Health at the Centers for Disease Control for their assistance during this project. Finally, we thank the anonymous in-state and out-of-state physicians who served as case reviewers for this project.

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Laparoscopic diagnosis and repair of spigelian hernia: Report of a case and technique

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Spigelian hernia is an uncommon hernia of the abdominal wall. We report a case of acquired spigelian hernia diagnosed and treated by laparoscopy. With more extensive use of laparoscopy to evaluate abdominal pain, the diagnosis of spigelian hernia can be made and repair accomplished without need for open exploratory surgery. (Am J Obstet Gynecol 1992;167:77-8.)

Key words: Spigelian hernia, laparoscopy, abdominal wall defect

Spigelian hernia is an uncommon defect of the abdominal wall. Bogojavlensky introduced laparoscopic treatment of inguinal and femoral hernias by preperitoneal patch repair at the 1989 meeting of the American Association of Gynecological Laparoscopists. In 1990 Popp[1] published a case of endoscopic patch repair in a female patient. We report the first case of spigelian hernia diagnosed and treated by laparoscopic techniques.

Case report

A 72-year-old woman was evaluated for a complaint of splitting lower abdominal pain of many years' duration, which had become progressively worse. She first noticed the pain about 6 months after abdominal hysterectomy performed in 1968 through a midline incision. In 1967 she had a tubal ligation through a minilaparotomy Pfannenstiel incision.

Physical examination outlined an area of tenderness along the left side of the abdominal wall. No fascial defect was felt. A small umbilical hernia was noted, which the patient stated was there from birth. A computed tomographic scan revealed a liver abnormality for which a liver biopsy was recommended.

Diagnostic approach. We performed laparoscopy to determine the source of the pain and to evaluate and perform a biopsy of the liver. The midclavicular line 2 cm below the right lowest rib margin was chosen as the puncture site for the 5 mm diagnostic laparoscope. This provided access to the liver for biopsy and for a full view of the umbilical hernia and lower pelvis.

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6/1/36680

The umbilical view revealed a 3 × 4 cm umbilical hernia defect with no adhesions. The infraumbilical region revealed a large omental adhesion herniating into a 10 cm defect in the semilunar line. The laparoscope was inserted into the gaping hernia. Palpation of the abdominal wall above the defect confirmed that the anterior abdominal wall was intact.

Surgical approach. Laparoscopic repair of the spigelian hernia required port sites for instrument insertion, which allowed triangulation and laparoscopic suturing of the defect: the midclavicular line 3 cm below the costal margin, 4 cm above the umbilicus in the midline, and 5 cm lateral to the umbilicus at that level.

The herniated omental tissues were bluntly and sharply dissected from within the defect. Bleeding was controlled with bipolar coagulation at 40 W. Once the defect was free of its omental contents, the abdominal wall was carefully inspected. No defect of the wall itself was visualized.

Repair of the hernia was then accomplished by laparoscopic placement of No. 1 Vicryl sutures on a CT 2 needle in an interrupted-Figure-eight pattern. Ten sutures effected complete closure. Ties were accomplished with the extracorporeal knotting procedure of Semm[2] with the Marlow knot pusher. The 45-degree, 5 mm Cook Ob/Gyn endoscopic needle driver (Kenh-053130) facilitated suturing on the anterior abdominal wall. The umbilical hernia was closed in a similar fashion.

Laparoscopic suturing on the anterior abdomen was facilitated by: (1) placement of suture and camera ports on the far contralateral side from the defect so the opposite anterior wall could be viewed and the needle delivered to the suturing site; (2) establishment and maintenance of 15 mm Hg pneumoperitoneum to assure a dome-shaped abdominal cavity; (3) external manipulation of the abdominal wall to bring both sides of the defect into full view; (4) use of the 45-degree-angle Cook needle driver to facilitate reverse acute-angle suturing; (5) suture lubrication to facilitate sliding each throw of the knot.

Comment

The hernia described by Andrian van der Spieghel (1578-1625) is one of the less common anterior abdominal wall hernias. The spigelian hernia occurs through congenital or acquired defects in the spigelian fascia of the transverse abdominis aponeurosis lying between the semilunar line and lateral edge of the rectus muscle. In 1764 Klinklosch named it *Spieghel's linear hernia.* Spangen detailed its anatomic aspects in 1976. In 1984 Spangen reviewed the published material on spigelian hernias and found that 744 patients had required surgery. No reports of laparoscopic repair have been made.

Many reports of spigelian hernia emphasize the difficulty in making the diagnosis. The nonspecific symptoms and intramural location are contributing factors.

Although uncommon, spigelian hernias are a source of abdominal pain. Many require emergency surgery. Laparoscopic evaluation of abdominal pain can allow diagnosis before laparotomy. With the technique described here spigelian hernias can be repaired laparoscopically and open exploratory surgery can be avoided.

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Oligohydramnios and megacolon in a fetus with vesicorectal fistula and anal-urethral atresia: A case report

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Severe oligohydramnios and extremely dilated bowel filled with hyperechogenic material floating in fluid were the ultrasonographic findings in a fetus at 27 weeks' gestation. Vesicorectal communication and urethral-anal atresia permitted urine to empty into the colon, causing megacolon, oligohydramnios, and markedly increased intraabdominal pressure resulting in pulmonary hypoplasia. (AM J OBSTET GYNECOL 1992;167:79-81.)

Key words: Oligohydramnios, fetal vesicorectal fistula, fetal megacolon, pulmonary hypoplasia

Malformations of the urogenital sinus are often associated with oligohydramnios and cystic dilatation of various segments of the urinary tract.

We present the first case to our knowledge that describes the prenatal ultrasonographic findings of a fetus with anal-urethral obstruction and spontaneous decompression of the urinary tract into the colon through a vesicorectal fistula. Autopsy findings were helpful in making the clinical-pathophysiologic correlations.

Case report

A 21-year-old primigravid woman was referred to our prenatal diagnostic center at 27 weeks' gestation because of severe oligohydramnios. She was exposed to parvovirus B-19 during the first trimester of her pregnancy. No rash or fever was noted, yet laboratory tests showed elevated maternal serum parvovirus B-19 IgG antibodies and low levels of immunoglobulin M, consistent with recent exposure or infection.

The ultrasonographic examination showed severe oligohydramnios, elevated diaphragms, compressed chest, and markedly distended abdomen and bowel. The abdominal cavity was completely occupied by a large cystic structure containing fluid and hyperechogenic substance (Fig. 1). Peristalsis-like waves were noted to be present within the mass. Kidneys and bladder appeared normal, and no other abnormalities were identified. The differential diagnosis included the conditions that are known to cause fetal intraabdominal calcifications. Meconium peritonitis was the leading consideration, although the floating nature of the hyperechogenic material was atypical. Other possibilities were tumors (hemangioma, hemangioendothelioma, hepatoblastoma, teratoma, and metastatic neuroblas-

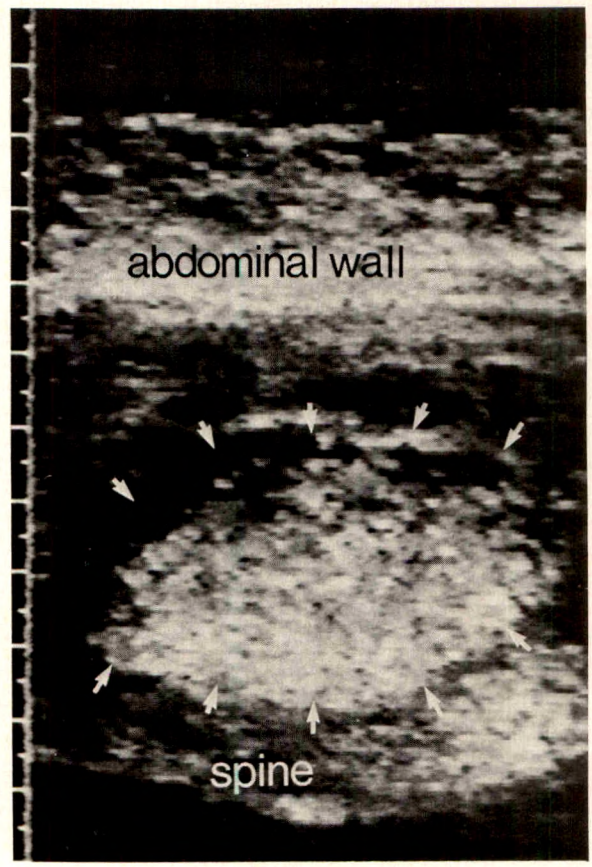


Fig. 1. Ultrasonography of fetal abdomen. *Arrows* show outline of distended bowel filled with floating hyperechogenic material.

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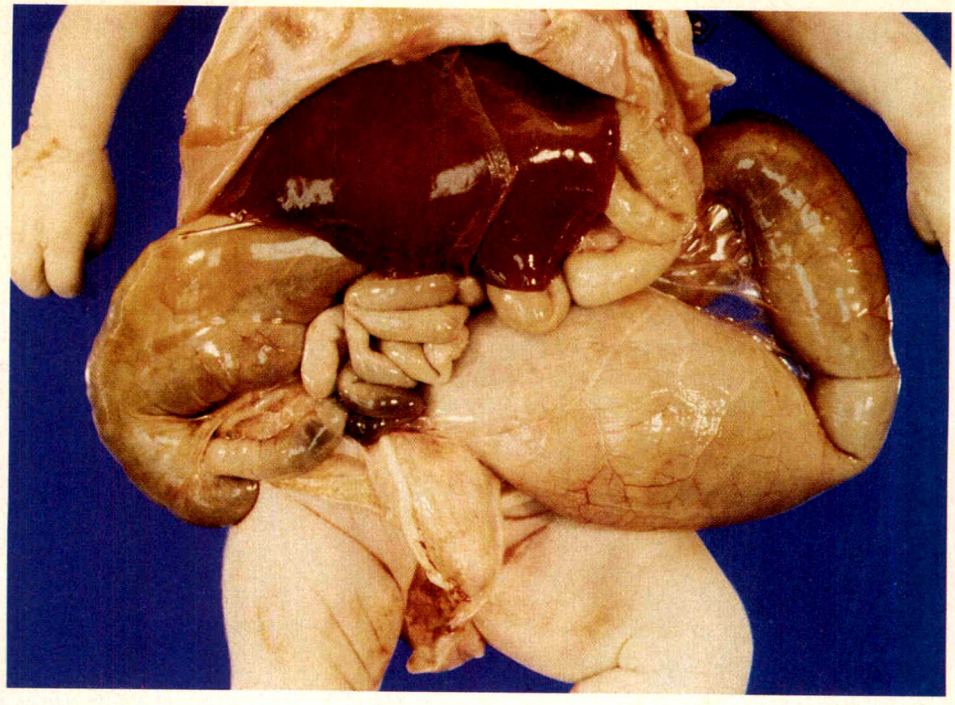


Fig. 2. Autopsy findings of extremely distended colon in association with urethral obstruction and vesicorectal fistula.

toma) and congenital infection (toxoplasmosis, cytomegalovirus). In an attempt to establish the diagnosis the patient was offered amniocentesis and cordocentesis for karyotype determination, as well as culture and serologic testing for cytomegalovirus, toxoplasmosis, and parvovirus. It was also intended to evaluate intestinal isozymes of alkaline phosphatase in amniotic fluid, as well as to perform molecular genetics studies for diagnosis of cystic fibrosis. However, the patient declined any invasive diagnostic procedures.

Serial ultrasonographic examinations continued to show the large echogenic abdominal mass and severe oligohydramnios. At 36 weeks' gestation she was admitted to the hospital in active labor. A 2650 gm male infant was delivered by emergency cesarean section for fetal distress. Apgar scores were 1 and 3 at 1 and 5 minutes, respectively. The infant was noted to have Potter's facies, lower-extremity deformation, markedly distended abdomen, imperforate anus, and ambiguous genitalia. Soon after delivery the infant was intubated and ventilated for respiratory insufficiency. Resuscitation was pursued after an episode of cardiac arrest, but the infant eventually died 4 hours after birth.

The final pathologic diagnoses included pulmonary hypoplasia, most likely as a secondary effect of the extremely distended colon (Fig. 2) and oligohydramnios. The diaphragms were elevated to the level of the third rib bilaterally. The colon was filled with watery, flocculent, yellowish green material identified as a mixture of meconium and urine. A fistula was identified between the distal bladder–proximal urethra to the rectum, and anal-urethral atresia was noted. Normal kidneys and ureters, normal penis, and undescended left testes were seen. Neonatal blood revealed a normal 46,XY karyotype.

Comment

During embryogenesis the cloaca is normally divided by a sheet of mesenchyme, the urorectal septum, into the urogenital sinus anteriorly and the rectum and upper anal canal posteriorly.[1] This process is completed by 6 weeks' gestation. In some cases, like the one presented, incomplete separation of the cloaca by the urorectal septum occurs. This type of malformation consists of anorectal agenesis with a fistula to the proximal urethra in male fetuses or the vagina in female fetuses. The prevalence of congenital abnormalities of the anus and rectum in general is approximately 1 in 5000 newborns. However, the prevalence of anorectal agenesis is even less. The associated urethral atresia in this case is more unique. It caused a diversion of urine into the colon and led to lethal complications, such as oligohydramnios and hypoplastic lungs.

This case presented a diagnostic dilemma to the perinatologist and the pediatric surgeon. This entity should be added to the differential diagnosis of fetal

abdominal mass as seen by ultrasonography. The ultrasonographic findings were hyperechogenic material floating in an abdominal mass and severe oligohydramnios in the presence of normal-appearing kidneys, ureters, and bladder. Persistent vesicorectal communication with anal and urethral atresia permitted urine to empty into and distend the entire colon. The mixture of meconium and urine produced the ultrasonographic picture of hyperechogenic material floating in fluid. The diversion of urine protected the kidneys and ureters from obstructive dysplasia and hydronephrosis-hydroureter. However, severe oligohydramnios, elevated diaphragms, and thoracic compression probably led to pulmonary hypoplasia and neonatal death.

This malformation was considered a sporadic event not related to a genetic disorder, infectious etiology, or specific teratogens. There does not appear to be a temporal relationship between the documented maternal parvovirus infection and the malformation that led to lethal secondary consequences. There have been no reports of such an association.[2]

Prenatal recognition of this entity at an early stage might permit operative urinary diversion. In theory, urine diverted into the amniotic cavity could prevent oligohydramnios, bowel distention, or pulmonary hypoplasia.

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Pathways to hysterectomy: Insights from longitudinal twin research

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OBJECTIVE: We hypothesized that genetic influences act on "liability" to hysterectomy, that secular influences might differentially affect relative importance of genetic and environmental influences, and that the sources of genetic influences could be identified from reported risk factors.

STUDY DESIGN: Hysterectomy data from an Australia-wide volunteer sample of female adult monozygotic and dizygotic twins are reported. In 1980 through 1982 a mailed questionnaire was completed by 1232 monozygotic female twin pairs and 751 dizygotic female twin pairs (3966 women) from the Australian Twin Register (wave 1). The same twins were surveyed by questionnaire 8 years later (wave 2).

RESULTS: A total of 366 had undergone hysterectomy by wave 1 and a further 198 at wave 2. The twin-pair correlations for liability to hysterectomy at wave 1 (0.61 ± 0.06 for monozygotic and 0.20 ± 0.11 for dizygotic) and wave 2 (0.65 ± 0.05 for monozygotic and 0.32 ± 0.09 for monozygotic) indicated a substantial genetic contribution. Reported risk factors accounted for only 15% of total variance.

CONCLUSION: Genetic influences on liability to hysterectomy were substantial and stable across birth cohorts, but the important sources of genetic influence on liability to hysterectomy are yet to be identified.

(Am J Obstet Gynecol 1992;167:82-8.)

Key words: Hysterectomy, twins, genetic influences

Hysterectomy has been the subject of considerable recent investigation as a result of the need to distinguish "real" clinical indications for the procedure from service provision distortions arising from health financing systems. In Australia the incidence of hysterectomy peaked in 1978,[1] with an age-standardized rate of 460 per 10^5,[2] compared with the United States peak in 1975.[3] The 1973 rate in the United States was 651 per 10^5 women, still significantly higher than the age-standardized Australian rate, which had declined to 376 per 10^5 by 1983. There is less variation in rate between Australian states than within the United States.[2]

Investigation of patient characteristics has found variance to be associated with factors such as race, education, and parity.[4] The role of patient demand in influencing hysterectomy rates in Australia has been noted,[5] and high prehysterectomy levels of psychiatric symptoms have been reported.[6, 7] To investigate further the causes of hysterectomy in Australian women, this prospective two-wave study of twins sought to identify the relative roles of environmental and genetic factors on "liability" to hysterectomy and the extent to which these are modulated through known risk factors. Twins discordant for hysterectomy, particulary monozygotic pairs, offer the ultimate matched-pair design to detect the effect of independent variables measured before the operation, and we have taken advantage of the 106 discordant pairs in our study for this purpose. We also investigated whether genetic effects could be detected on the age at which hysterectomy was performed in pairs concordant for hysterectomy.

Methods

Wave 1 (1980 through 1982). In 1980 through 1982, as part of a health survey by mailed questionnaire, information about hysterectomy was obtained from 1983 female twin pairs in a large sample of 3808 adult twin pairs from the Australian National Health and Medical Research Council Twin Register.[8-10] Questionnaires were mailed to 5967 twin pairs aged ≥18 years. Ages of respondents then ranged from 18 to 88 years. After one or two reminders to nonrespondents, completed questionnaires were returned by both members of 3808 twin pairs (64% pairwise response rate). A two-item zygosity questionnaire was used to determine zygosity for same-sex pairs.[8] Such questionnaires have been shown to give at least 95% agreement with diagnosis on the basis of extensive blood typing.[11, 12]

Twins who were menstruating regularly at the time of survey were asked about cycle length, duration of

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menstrual periods, flow, pain, and limitation related to menstruation. Twins who had stopped menstruating were asked whether this was due to hysterectomy, menopause, or some other cause and at what age it occurred. Wave 1 variables tested for association with subsequent hysterectomy status were oral contraceptive use and duration of use, age at menarche, history of pregnancy, total pregnancies, age at first pregnancy, terminations of pregnancy, days of menstrual bleeding, self-ratings (three-point scales) of heaviness of menstrual flow, menstrual pain, and whether menstruation was limiting, average menstrual cycle length, and number of obstetric difficulties experienced, DSSI/sA (Delusions-Symptoms-States Inventory/States of Anxiety and Depression) anxiety and depression scores,[13] Extraversion and Neuroticism scores,[14] educational attainment, and history of smoking.

Wave 2 (1988 through 1990). Between 1988 and 1990, twin pairs who had responded to the wave 1 survey were traced and relocated for follow-up. The second questionnaire replicated many of the earlier questions, including that on hysterectomy, and included some new items on premenstrual syndrome and other potential risk factors. This two-wave design allowed us to consider wave 1 variables prospectively as possible "risk factors" for subsequent hysterectomy. Wave 2 variables tested for association with hysterectomy were premenstrual symptom interference in usual activities, self-diagnosis of "premenstrual syndrome or premenstrual tension," whether treatment had ever been sought for premenstrual problems, perception of own social class, total Parental Bonding Instrument (PBI) score[15] and PBI factor scores on parental coldness, overprotection, and discouragement of child's autonomy, and total number of life events from a series of items including experience of rape or sexual assault and loss of a pregnancy or a child.

Twin pair correlations. We assume that hysterectomy is performed when a threshold is crossed on an underlying continuum of symptoms. These symptoms have many (multifactorial) causes; therefore it is reasonable to assume that the continuum is normally distributed. It is much more informative to consider the correlation between relatives for the continuous trait, or *liability*, than for the simple dichotomy of presence or absence of hysterectomy. The appropriate statistic that estimates this correlation in liability between twins is the *polychoric correlation*, of which the special case for two dichotomous variables is the tetrachoric correlation. These may be calculated with the PRELIS 1.12 software system.[16]

Results

Response. Responses to wave 1 were received from 1983 complete female pairs (1232 monozygotic and 751 dizygotic), and 1448 (921 monozygotic and 527 dizygotic) also answered the hysterectomy question at wave 2 (76% of pairs where both twins were still alive). Of the 366 women reporting hysterectomy at wave 1, 339 also completed the wave 2 survey. A total of 564 hysterectomies was reported over the two waves (366 at wave 1 and a further 198 at wave 2), 475 being in responding pairs at wave 2 (319 monozygotic and 156 dizygotic pairs). Pairwise concordance for hysterectomy for twins born before 1947 in wave 1 was 34.1% for monozygotic twins and 15.7% for dizygotic twins. At wave 2, for twins born before 1955, pairwise concordance was 39.4% for monozygotic twins and 21.4% for dizygotic twins. At wave 1 only 17 hysterectomies were reported by women born after 1946, and at wave 2 only five were reported by women born after 1954.

Twins who had undergone hysterectomy were asked in both questionnaires how old they were when they had the operation and in the wave 2 questionnaire whether the hysterectomy was before, during, or after menopause and whether both ovaries were removed. The mean ± SD for age at hysterectomy in wave 2 was 40.4 years ± 7.9, with a range of 18 to 76 years. Most hysterectomies were performed between the ages of 34 and 43 years (53%), so most women (77%) underwent hysterectomy before menopause.

Survival analysis. Hysterectomy increases in incidence with age but, as previously discussed, prevalence has also varied for secular reasons including changes in surgical fashion and health financing. Both these temporal effects may differentially affect the importance of genetic and environmental influences on hysterectomy, and it is important to try to disentangle them. Fortunately, the two-wave design of our study gives some scope for this. It is also important to ensure that any such temporal effects are similar in the monozygotic and dizygotic subsamples.

Survival to age at hysterectomy was calculated for twins in the two study waves. The most powerful test of secular change in hysterectomy rates was comparison of women aged ≥40 in wave 1 (born before 1940) with those in the same age group at wave 2 (born before 1948). There was overlap in the two samples, because women born before 1940 who responded to wave 2 were also in the second sample. Nevertheless, we see a marked secular increase in hysterectomy rates (Fig. 1) in the wave 2 sample (Lee-Desu $\chi^2_1 = 9.49$, $p = 0.002$). There was no heterogeneity in survival to hysterectomy between older (>40 years) monozygotic and dizygotic twins at wave 1 ($\chi^2_1 = 2.76$, $p = 0.10$) or wave 2 ($\chi^2_1 = 0.89$, $p = 0.35$).

Twin correlations. Because hysterectomy incidence varies with age and also for secular reasons, we tabulated the tetrachoric correlations (and their asymptotic standard errors) for liability to hysterectomy in monozygotic and dizygotic twins for three age bands (34 to 40, 41 to 48, and >48 years) and for the total sample

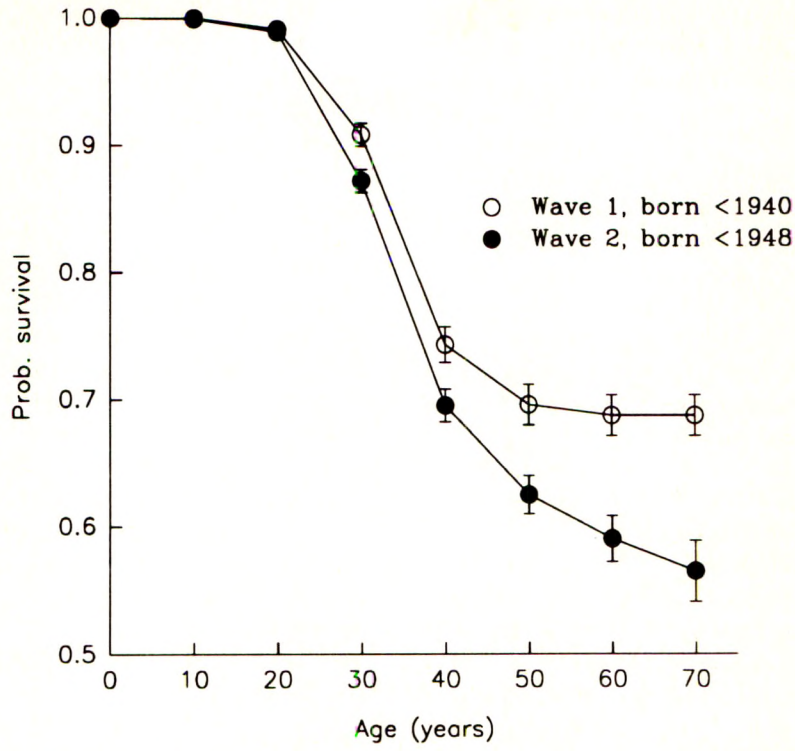


Fig. 1. Probability of reaching given age without hysterectomy in women aged >40 in waves 1 and 2.

Table I. Concordance for presence or absence of hysterectomy and twin pair correlations in liability by zygosity, age group, and study wave

Wave No.	*Born*	*Monozygotic*					*Dizygotic*				
		H^-H^-	H^+H^-	H^+H^+	*Total*	$r \pm SE$	H^-H^-	H^+H^-	H^+H^+	*Total*	$r \pm SE$
1	pre-1932	148	59	35	242	0.60 ± 0.08	79	57	9	145	−0.04 ± 0.15
2	pre-1940	126	79	65	270	0.58 ± 0.07	68	57	18	143	0.15 ± 0.14
1	1932-1939	77	34	18	129	0.54 ± 0.12	45	19	5	69	0.31 ± 0.22
2	1940-1947	111	30	14	155	0.62 ± 0.11	58	28	7	93	0.28 ± 0.19
1	1940-1946	144	17	4	165	0.57 ± 0.17	96	15	3	114	0.46 ± 0.21
2	1948-1954	170	28	10	208	0.62 ± 0.12	104	14	2	120	0.38 ± 0.24
TOTAL											
1	pre-1947	369	110	57	536	0.61 ± 0.06	220	91	17	328	0.20 ± 0.11
2	pre-1955	407	137	89	633	0.65 ± 0.05	230	99	27	356	0.32 ± 0.09

H^-H^-, Hysterectomy absent in both twins; H^+H^-, hysterectomy present in one twin and absent in the other; H^+H^+, hysterectomy present in both twins.

(aged >33 years) in both waves of the study (Table I). The first point to notice is that correlations are remarkably consistent between waves for the same age band, and this gives us confidence that secular changes in incidence have little effect on twin correlations. Second, the monozygotic correlations are remarkably consistent (around 0.6) in all age bands. In contrast, the dizygotic correlations appear to increase from the oldest to the youngest age band, although the standard errors are large.

Pearson correlations for age at hysterectomy within pairs concordant for hysterectomy at wave 2 suggested that genetic influence might be operating on the timing of hysterectomy ($r = 0.49 \pm 0.11$ for monozygotic and $r = 0.15 \pm 0.22$ for dizygotic), as well as on "liability" to hysterectomy. This was not apparent at wave 1, because monozygotic and dizygotic correlation coefficients were of equal magnitude ($r = 0.50 \pm 0.14$ for monozygotic and $r = 0.50 \pm 0.25$ for dizygotic), suggesting that variation might be due to environmental influences only. Note, however, that numbers are small (Table I) and that there were only 17 concordant dizygotic pairs at wave 1.

Model fitting to test for heterogeneity. The decline

in the dizygotic correlation for hysterectomy liability with increasing age suggests that the relative importance of genetic and environmental influences may also vary with age, genetic factors becoming more important in older women. To test this formally, we used LISREL 7.16[17] software to fit models of variation simultaneously to monozygotic and dizygotic correlations for the three age bands. Variance disaggregates into three components: additive gene action *(h)*, environmental influences specific to the individual *(e)*, and either environmental effects common to both cotwins *(c)* or nonadditive gene action *(d)* (for example, dominance or epistasis).

Fitting the simplest model consistent with the observed correlations (comprising only h and e) to the three cohorts in wave 1 resulted in a very good fit ($\chi^2_5 = 5.47$). Adding shared environment ($\chi^2_4 = 5.47$) or nonadditivity ($\chi^2_4 = 4.41$) did not significantly improve fit, and dropping gene action from the model (c and e, no h) significantly worsened fit ($\chi^2_5 = 14.88$, $p = 0.011$). Also with wave 2, models fitted to the three birth cohorts simultaneously resulted in a best-fitting simple model comprising h and e ($\chi^2_5 = 1.35$). Again, fit significantly worsened when h was dropped (ce model $\chi^2_5 = 11.09$, $p = 0.05$). These results are not consistent with the existence of heterogeneity in genetic and environmental effects between older and younger twins. Estimates of heritability of liability were consistent across study waves ($h^2 = 0.56$ for wave 1 and $h^2 = 0.59$ for wave 2), affirming that genetic predisposition to hysterectomy was stable and not influenced by changing environmental influences. Any significant influence of shared environment was also rejected.

Covariates of hysterectomy. Polychoric correlations were computed between the potential risk factors in Methods and post–wave 1 hysterectomy. Wave 2 reports of whether premenstrual symptoms ever interfered "with work, daily activities, usual social events or . . . relationships with others" (retrospective) were the most highly correlated with hysterectomy. All significant covariates are presented in Table II. Hypotheses concerning preexisting psychologic morbidity and experiences of loss in women undergoing hysterectomy were not supported by our data.

Twins discordant for hysterectomy. Discordant twin pairs, especially monozygotic pairs, provide an ideal case-control design. Variables hypothesized as precursors of hysterectomy were tested for the 106 twin pairs discordant for "new" hysterectomy, where one twin had a hysterectomy after the wave 1 survey and her cotwin had not had a hysterectomy by wave 2 (see Table III). "New" hysterectomy was selected because the two-wave design allowed for characteristics reported at wave 1 to be reported independently of the subsequent operation. We performed t tests on continuous variables. Fisher's exact test was used on contingency tables to detect significant differences in rates of endorsement of extreme values of categoric variables (for example, "very painful" menses vs "moderately painful" or "no trouble").

Table II. Significant wave 1 covariates* of subsequent hysterectomy in twins born before 1955

Covariate	*r*
1. Premenstrual syndrome symptoms interfered ever†	0.28
2. Limitation by menstruation	0.23
3. Menstrual flow	0.22
4. Average length of menses (days of bleeding)	0.19
5. Menstrual pain (dysmenorrhea)	0.17
6. No. of obstetric problems	0.15
7. Length of average menstrual cycle	−0.08
8. Neuroticism score	0.06
9. Education level	−0.05

*Sample size of 1530 to 2263. All correlations significant at $p < 0.001$ level except 7 and 8 ($p < 0.01$) and 9 ($p < 0.05$).
†Reported retrospectively at wave 2.

In discordant pairs, liability to hysterectomy was associated with the extreme category of "very painful" periods (odds ratio 9.99, 95% confidence interval 2.58 to 38.89). Also risk factors, but to a lesser extent, were the extreme categories of retrospectively reported interference of premenstrual symptoms in usual activities ("a lot") (odds ratio 5.64, 95% confidence interval 1.70 to 18.68), having ever suffered from premenstrual syndrome (odds ratio, 2.04, 95% confidence interval 1.05 to 4.05), previous "heavy" menstrual flow (odds ratio 2.19, 95% confidence interval 1.04 to 4.58), and having ever sought treatment for premenstrual syndrome (odds ratio 2.08, 95% confidence interval 1.00 to 4.41). Reporting "very limiting" periods (odds ratio 3.24, 95% confidence interval 0.74 to 14.15) was not a significant risk factor.

Multivariate path analysis. To see how much of the variance in liability to hysterectomy could be explained by these covariates, a simple multivariate path model was fitted with LISREL software (Fig. 2). The influence of particular variables may appear inconsistent with the odds ratios reported previously because the highest odds ratios were found for the rarest events (i.e., those with least frequent endorsement) and in the path analysis the entire distributions are entered. Our path model had the advantage of specifying one-way effects from the possible risk factors to the underlying risk of hysterectomy and ipso facto of addressing the latent variable rather than only the observed event. We were unable to explain satisfactorily the basis of the genetic variance in liability to hysterectomy by identifying genetic and environmental influences on reported risk factors. The major covariates of hysterectomy measured in our study accounted for only 15% of the phenotypic variance in liability to hysterectomy. Cholesky

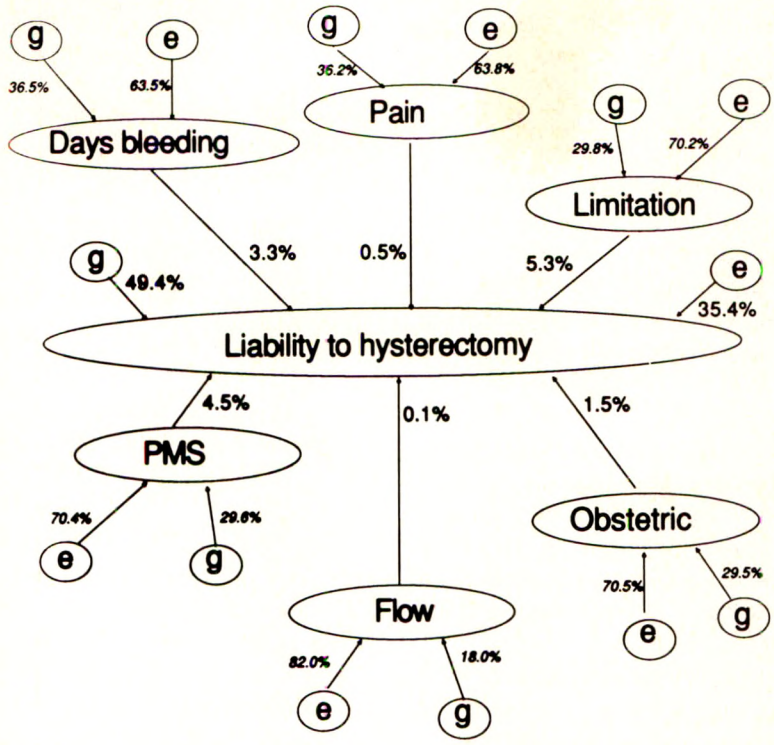


Fig. 2. Path model for causes of variation in liability in hysterectomy. Genetic and unique environmental sources of variation specific to each variable are denoted *g* and *e*. Paths between measured variables denote direct phenotypic influence. Percentages on paths indicate percent of variance in target variable contributed by each source.

saturation of the model to allow for all intercorrelations[18, 19] similarly revealed no notable genetic loading of hysterectomy on other risk factors but considerable environmental loading (data not shown). Thus genetic influences on liability to hysterectomy can be ascribed only in small part (perhaps 10%) to genetic influences on identified risk factors, so the major heritable factors are yet to be identified.

Comment

We have found clear evidence of genetic influences on liability to hysterectomy. These influences are stable across birth cohorts in which there are differences in incidence because of both aging and secular changes in surgical practice and health financing. Our analysis of reported risk factors has not revealed the basis of the largest part (85%) of the variance in liability to hysterectomy, and at least half of this is due to unidentified genetic factors. Extent of perceived interference of premenstrual problems, accounting for <5% of variance, was ahead of other menstrual dysfunction variables as risk factors for hysterectomy. The personality trait Neuroticism was barely a significant covariate and certainly played no notable role in explaining the variance. Measures of depression and anxiety proved to have no explanatory relevance. Our findings do not support the important role of these psychologic dimensions in risk for hysterectomy claimed by some authors.[6, 7]

Central to our interpretation of higher monozygotic than dizygotic twin correlations as evidence for genetic influence on hysterectomy liability is the assumption that monozygotic twins experience environmental treatments no more similarly than their dizygotic counterparts, at least insofar as these treatments pertain to the trait in question, in this case hysterectomy. This "equal environments assumption" has been challenged on the grounds that there is indeed a higher "environmental" correlation between monozygotic than dizygotic twin pairs. Evidence suggests, however, that any such effect arises because monozygotic twins, being genetically identical, create more similar environments for themselves.[20-22] If some monozygotic twins were becoming concordant for hysterectomy from frivolous reasons of imitation rather than genuine medical need, one might expect the prevalence to be higher in monozygotic than dizygotic twins; however, as our survival analysis shows, there is no significant difference in the rates of the two zygosity groups. This does not support the possibility either that physicians might have treated adult dizygotic twins (as individuals) differently from monozygotic twins or that monozygotic twin pairs were

Table III. Significant differences in pairs who became discordant for hysterectomy between wave 1 and wave 2

	Discordant pairs (H^+H^-)								
	Monozygotic (66 pairs)			*Dizygotic (40 pairs)*			*Monozygotic plus dizygotic (106 pairs)*		
	H^+	H^-	p	H^+	H^-	p	H^+	H^-	p
"Very painful" menses (%)	28.6	4.1	0.001	16.7	0.0	0.026	24.1	2.5	0.000
Days of bleeding	5.9	4.9	0.001	5.5	4.8	0.071	5.7	4.9	0.000
Premenstrual syndrome interfered "a lot" (%)	24.3	6.1	0.009	12.1	0.0	0.057	19.5	3.8	0.001
"Heavy" flow (%)	34.0	22.6	0.075	30.0	10.0	0.042	32.5	18.1	0.015
"Premenstrual syndrome" suffered (%)	72.9	62.5	0.096	72.7	48.5	0.027	72.8	56.8	0.014
Treatment for premenstrual syndrome (%)	34.7	24.5	0.096	28.1	9.4	0.042	32.1	19.8	0.020
Menses "very limiting" (%)	10.3	5.4	0.237	10.3	0.0	0.118	10.3	2.9	0.066
Years of using oral contraceptives	4.1	3.7	0.473	4.1	5.9	0.023	4.1	4.5	0.333

H^+, Hysterectomy present; H^-, hysterectomy absent.

more likely to be referred to the same specialist as dizygotic twin pairs. Similarly, the argument that, if the increased dizygotic twinning in older mothers is genetically influenced, there might be selection against earlier hysterectomy in dizygotic twins compared with monozygotic twins is not supported by the similar age-at-hysterectomy distributions in the two zygosity groups.

Limitations of the study demanding acknowledgement are: (1) data censoring, (2) validity of self-reporting, and (3) representativeness of the sample. Using twin pairs discordant for recent hysterectomy increases the likelihood of the cotwin having a future (and yet-to-be-reported) hysterectomy after wave 2. Analysis of hysterectomy, like analysis of many procedures, events, or illnesses of importance to epidemiologists, is influenced by the problem of timing of onset, leading to data censoring.[23] Only if subjects were assessed at death could the exact lifetime prevalence of hysterectomy be known. The approach of Neale et al.[24] will be used in future analyses to deal with the problem, which has also arisen in analyses of appendectomy[25] and tonsillectomy[26] twin data.

All variables were measured by self-report; hysterectomy was not validated by the twin in person, her cotwin, or a medical practitioner. For most women, however, hysterectomy would be a noteworthy event requiring hospitalization and therefore would likely be more accurately reported than less well-defined medical conditions. The wave 1 questionnaire actually stated that hysterectomy was "removal of the womb." In support, none of the twins who answered "no" to hysterectomy at wave 2 had said "yes" at wave 1. When age at hysterectomy was reported at both waves, the Pearson correlation for the two reports was $r = 0.94$ ($p < 0.001$), suggesting acceptable consistency in reporting over an average of 8 years.

The twin sample has been shown to be representative of the Australian population on a number of variables, such as drinking behavior,[27] personality factors, and anxiety and depression.[8, 20] The wave 1 sample was unselected for anything except volunteering to enroll in the Australian National Health and Medical Research Council Twin Register and returning the questionnaire. On the basis of age-specific hysterectomy rates in the Australian State of New South Wales in 1983, the expected cumulated total proportion of women having lost a uterus was 29% (25% for a 65-year-old woman).[2] Our cumulated risk of hysterectomy in the wave 1 sample (1980 through 1982) was 31% at age 65, a higher but comparable proportion. At wave 2 the cumulated risk was 41% at age 60, reaching a maximum of 43%, which is higher than might be expected from 1983 projections, although the modal year of wave 2 data collection was 1989, for which we have no normative data and numbers were relatively small in these older age groups.

We intend to include medical and pathology records relating to hysterectomy in further analysis to help elucidate the sources of genetic influence. We need to compare the distribution of indications for the hysterectomies in monozygotic and dizygotic twins. Clinical symptoms presented to twins' medical practitioners may have differed from the self-reported characteristics assessed in this study. Hysterectomy may have followed wave 1 reports by between 1 and 8 years; therefore there may be considerable heterogeneity in our sample in the relationship with antecedent "risk factors." Lack of consensus concerning recognition and significance of medical indications may make retro-

spective interpretation difficult.[3] Nevertheless, we plan to gather information on indications and the twins' reasons for hysterectomy in a new phase of the study. The power of our findings will be further tested by analysis of data being collected on hysterectomy from these twins' mothers, sisters, and adult daughters and from female relatives of male twins in the Australian Twin and Family Study.

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NORPLANT® SYSTEM
levonorgestrel implants

Lasts 5 years...yet is reversible

Serious as well as minor side effects may occur. The most common side effect which has been observed is menstrual bleeding irregularities.

Please see brief summary of prescribing information on the following page.

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NORPLANT® SYSTEM
levonorgestrel implants
Lasts five years…yet is reversible

BRIEF SUMMARY OF PRESCRIBING INFORMATION. CONSULT THE PACKAGE LITERATURE FOR FULL PRESCRIBING INFORMATION.

Indications and Usage

The NORPLANT SYSTEM is indicated for the prevention of pregnancy and is a long-term (up to 5 years) reversible contraceptive system. The capsules should be removed by the end of the 5th year. New capsules may be inserted at that time if continuing contraceptive protection is desired.

Contraindications

1. Active thrombophlebitis or thromboembolic disorders. 2. Undiagnosed abnormal genital bleeding. 3. Known or suspected pregnancy. 4. Acute liver disease; benign or malignant liver tumors. 5. Known or suspected carcinoma of the breast.

Warnings

A. WARNINGS BASED ON EXPERIENCE WITH THE NORPLANT SYSTEM

1. *Bleeding Irregularities* — Most women can expect some variation in menstrual bleeding patterns. Irregular menstrual bleeding, intermenstrual spotting, prolonged episodes of bleeding and spotting, and amenorrhea may occur, and could mask symptoms of cervical or endometrial cancer. Overall, these irregularities diminish with continued use. Because amenorrhea may occur, missed menstrual periods cannot serve as the only identifier of early pregnancy. Perform pregnancy tests whenever pregnancy is suspected. If pregnancy occurs, the capsules must be removed. Hemoglobin concentrations found in clinical trials generally indicated that reduced menstrual blood loss is associated with NORPLANT SYSTEM use. Blood loss resulting in hemoglobin values consistent with anemia occurred rarely.

2. *Delayed Follicular Atresia* — Atresia of the follicle is sometimes delayed, resulting in enlarged follicles that are clinically indistinguishable from ovarian cysts. In the majority of women, enlarged follicles disappear spontaneously. Rarely, they twist or rupture and surgical intervention may be required.

3. *Ectopic Pregnancies* — Ectopic pregnancies have occurred among NORPLANT SYSTEM users, although clinical studies have shown no increase in the rate of ectopic pregnancies per year among NORPLANT SYSTEM users as compared with users of no method or of IUDs. The incidence among NORPLANT SYSTEM users (1.3 per 1000 woman-years) was significantly below the rate estimated for noncontraceptive users in the U.S. (2.7 to 3.0 per 1000 woman-years). Ectopic pregnancy risk may increase with duration of NORPLANT SYSTEM use and increased weight of the user. Rule out ectopic pregnancy in any patient presenting with lower-abdominal pain.

4. *Breast-feeding* — Steroids are not the contraceptives of first choice for lactating women. Levonorgestrel has been identified in breast milk. Limited data suggests no significant effects on infant growth or health when mothers used the NORPLANT SYSTEM beginning 6 weeks after parturition.

5. *Foreign-body Carcinogenesis* — Rarely, cancers occur at foreign-body intrusion sites or old scars. None has been reported in NORPLANT SYSTEM clinical trials and risk to users is judged to be minimal.

6. *Thromboembolic Disorders* — Remove capsules if active thrombophlebitis or thromboembolic disease develops. With prolonged immobilization removal should be considered.

B. WARNINGS BASED ON EXPERIENCE WITH COMBINATION (PROGESTIN PLUS ESTROGEN) ORAL CONTRACEPTIVES (OCs)

Note: Many of the side effects or risks listed below are thought to be estrogen-related; the association of the NORPLANT SYSTEM progestin-only method to these risks is unknown.

1. *Cigarette Smoking* — Cigarette smoking increases the risk of serious cardiovascular side effects from combined OC use. Risk increases with age and heavy smoking (≥15 cigarettes/day) and is quite marked in women over 35 years old.

2. *Elevated Blood Pressure* — Increase in blood pressure has been reported in combination OC users; prevalence increases with long exposure.

3. *Thromboembolic Disorders and Other Vascular Problems* — An increased risk of thromboembolic and thrombotic disease is associated with combination OC use. Estimate of relative risk is 4- to 11-fold higher for users vs. nonusers.

Cerebrovascular Disorders: Combination OCs increase the relative and attributable risk of cerebrovascular events (thrombotic and hemorrhagic strokes). Generally, risk is greatest among hypertensive women > 35 years of age who smoke.

Myocardial Infarction (MI): An increased risk of MI has been attributed to combined OC use. This is thought to be primarily thrombotic in origin and related to the estrogen component. Increased risk occurs primarily in smokers or women with other underlying risk factors for coronary-artery disease. Relative risk of heart attack for combined OC users is estimated as 2 to 6 times that for nonusers. Absolute risk is very low for women under 30 years old.

Studies indicate a significant trend toward higher MI and stroke rates with increased progestin doses in combination OCs. However, recent data indicated no increased MI risk with past use of levonorgestrel-containing OCs.

4. *Carcinoma* — Recent evidence in the literature suggests no association between OC use and increased risk of breast cancer in the overall population of users. The Cancer and Steroid Hormone (CASH) study also showed no latent effect on breast cancer risk for at least a decade following long-term use. Some of these same studies have shown an increased relative risk of breast cancer in certain subgroups; no consistent pattern has been identified. Some studies suggest an association between combination OCs and an increase in the risk of cervical intra-epithelial neoplasia in some populations of women. The extent to which such findings may be due to differences in sexual behavior and other factors remains controversial. A cause-and-effect relationship between combined OC use and breast or cervical cancer has not been established. Combination OCs may decrease ovarian and endometrial cancer risk. Irregular bleeding patterns associated with NORPLANT SYSTEM use could mask cervical or endometrial cancer symptoms.

5. *Hepatic Tumors* — Hepatic adenomas are associated with combination OC use; estimated incidence is 3 events per 100,000 users per year. Risk increases after 4 or more years of use. Hepatic adenomas are benign but may rupture and cause death through intra-abdominal hemorrhage.

6. *Ocular Lesions* — Retinal thrombosis is associated with OC use and is believed to be related to the estrogen component. However, NORPLANT SYSTEM capsules should be removed if there is unexplained partial or complete vision loss; onset of proptosis or diplopia; papilledema; or retinal vascular lesions. Undertake appropriate diagnostic and therapeutic measures immediately.

7. *Use Before or During Early Pregnancy* — Extensive epidemiological studies reveal no increased risk of birth defects when OCs are used prior to pregnancy. Studies also do not suggest a teratogenic effect when taken inadvertently during early pregnancy. No evidence suggests that risk with NORPLANT SYSTEM use is different.

8. *Gallbladder Disease* — Early studies reported an increased lifetime relative risk of gallbladder surgery in OC or estrogen users. More recent studies, however, indicate that the relative risk of gallbladder disease with OC use may be minimal; this may be related to use of OCs with less estrogen and progestin content.

Precautions

GENERAL:

1. *Physical Examination and Follow-up* — A complete medical history and physical examination should be taken prior to implantation or reimplantation of NORPLANT SYSTEM capsules and at least annually during its use. Exams should include special reference to the implant site, blood pressure, breasts, abdomen and pelvic organs, including cervical cytology and relevant laboratory tests. Rule out malignancy in cases of undiagnosed, persistent or recurrent abnormal vaginal bleeding. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care.

2. *Carbohydrate Metabolism* — Altered glucose tolerance is found in some combination and progestin-only OC users. Effects of NORPLANT SYSTEM on carbohydrate metabolism appear minimal. Observe diabetic and prediabetic patients carefully while using the NORPLANT SYSTEM. Follow women being treated for hyperlipidemias closely if using the NORPLANT SYSTEM. Some progestins may elevate LDL and may render control of hyperlipidemias more difficult. (See **Warnings**.)

3. *Liver Function* — Consider removing capsules if jaundice develops. Steroid hormones may be poorly metabolized in patients with impaired liver function.

4. *Fluid Retention* — Steroid contraceptives may cause some degree of fluid retention. Prescribe with caution, and careful monitoring, in patients with conditions possibly aggravated by fluid retention.

5. *Emotional Disorders* — Consider removing capsules if significant depression occurs since the symptom may be drug-related. Observe carefully those with history of depression and consider removal if depression recurs to a serious degree.

6. *Contact Lenses* — Contact-lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist.

7. *Insertion and Removal* — Insertion is advised during the first 7 days of the cycle or immediately following abortion to insure that the woman is not pregnant and to assure contraceptive effectiveness during first cycle of use. Capsules may be inserted at any time during the cycle provided pregnancy has been excluded and a nonhormonal contraceptive method is used for the remainder of the cycle. Insertion is not recommended before 6 weeks postpartum in breast-feeding women. Follow insertion and removal instructions closely. Healthcare professionals are strongly advised to be instructed in the procedures before they attempt them. Proper insertion just under the skin facilitates removals; proper insertion and removal should result in minimal scarring. If all capsules cannot be removed at first attempt, attempt removal later when the site has healed. Bruising may occur at implant site during insertion or removal. Hyperpigmentation may occur over implant site but is usually reversible following removal. **See Full Prescribing Information for Detailed Insertion/Removal Instructions.**

8. *Infections* — Implant site infection has been uncommon (0.7%); aseptic technique and proper insertion/removal reduces possibility of infection. Institute treatment if infection occurs; remove capsules if infection persists.

9. *Expulsion* — Expulsion of capsules was uncommon; frequency increased when capsule placement was extremely shallow, was too close to incision, or when infection was present. Replace expelled capsule with new sterile capsule. Treat and cure any infection before replacement. Contraceptive efficacy may be inadequate with fewer than 6 capsules.

10. *Provisions for Removal* — Advise women that capsules may be removed at any time for any reason. Personnel instructed in removal technique should perform removal on request or at the end of 5 years of usage. Upon removal, dispose of capsules in accordance with Centers for Disease Control Guidelines for biohazardous waste.

DRUG INTERACTIONS: Reduced efficacy (pregnancy) in NORPLANT SYSTEM users has been reported when phenytoin or carbamazepine were used concomitantly. Warn NORPLANT SYSTEM users of possible decreased efficacy with use of related drugs.

DRUG/LABORATORY TEST INTERACTIONS: 1. Sex-hormone-binding globulin concentrations are decreased. 2. Thyroxine concentrations may be slightly decreased and triiodothyronine uptake increased.

CARCINOGENESIS: See **Warnings** section and Full Prescribing Information.

PREGNANCY: Pregnancy Category X. See **Warnings** section and Full Prescribing Information.

NURSING MOTHERS: See **Warnings** section and Full Prescribing Information.

INFORMATION FOR THE PATIENT: See Patient Labeling. Provide copy of patient labeling to the patient. Advise patients that Prescribing Information is available upon request. Inform prospective users of risks and benefits associated with NORPLANT SYSTEM use, with other forms of contraception, with no contraception, and about insertion/removal procedures. Informed consent from all patients may be desired in light of techniques involved with insertion and removal.

Adverse Reactions

The following have been associated with the NORPLANT SYSTEM during first year of use: many bleeding days or prolonged bleeding (27.6%); spotting (17.1%); amenorrhea (9.4%); irregular (onsets of) bleeding (7.6%); frequent bleeding onsets (7.0%); scanty bleeding (5.2%); pain or itching near implant site - usually transient - (3.7%); infection at implant site (0.7%); removal difficulties affecting subjects - based on 849 removals - (6.2%).

Controlled clinical studies suggest that the following, occurring during the first year, are probably associated with NORPLANT SYSTEM use: headache; nervousness; nausea; dizziness; adnexal enlargement; dermatitis; acne; change of appetite; mastalgia; weight gain; hirsutism, hypertrichosis, and scalp-hair loss. The following were reported with a frequency of 5% or greater during the first year and possibly may be related to NORPLANT SYSTEM use: breast discharge; cervicitis; musculoskeletal pain; abdominal discomfort; leukorrhea; vaginitis.

Overdosage

Overdosage may cause fluid retention with its associated effects and uterine bleeding irregularities.

Dosage and Administration

The NORPLANT SYSTEM consists of six Silastic® capsules, each containing 36 mg of the progestin, levonorgestrel. The total administered (implanted) dose is 216 mg. Implantation of all six capsules should be performed during the first 7 days of the onset of menses by a healthcare professional instructed in the NORPLANT SYSTEM insertion technique. Insertion is subdermal in the midportion of the upper arm about 8 to 10 cm above the elbow crease. Distribution should be in a fanlike pattern, about 15 degrees apart, for a total of 75 degrees. Proper insertion will facilitate later removal. **(See Full Prescribing Information for Detailed Insertion/Removal Instructions.)**

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Maternal and fetal hemodynamic effects of autologous blood donation during pregnancy

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OBJECTIVES: Our objectives were to describe the maternal and fetal hemodynamic impact of a 1 U acute blood loss during pregnancy and to compare the hemodynamic responses of pregnant women with those of nonpregnant controls.

STUDY DESIGN: With Doppler techniques cardiac output and total peripheral vascular resistance were determined in 16 pregnant women in the third trimester and in 16 nonpregnant volunteers before and during orthostatic stress, before and after donation of 450 ml of whole blood. In pregnant women the fetal umbilical artery systolic/diastolic ratio was also determined. Data were subjected to multiple logistic regression analysis.

RESULTS: In both groups orthostasis provoked significant decreases in cardiac output and increases in total peripheral vascular resistance. The hemodynamic effects of blood loss were less pronounced and did not significantly change the hemodynamic response to orthostasis in either group. Neither orthostasis nor blood loss caused significant changes in the umbilical systolic/diastolic ratio.

CONCLUSION: These data support the hemodynamic safety of uncomplicated autologous blood donation during late pregnancy. (Am J Obstet Gynecol 1992;167:89-93.)

Key words: Hemodynamics, autologous blood donation, pregnancy

The precise hemodynamic impact of a 1 U acute blood loss on systemic and fetoplacental perfusion has not been described in normal human pregnancy or in nonpregnant individuals. Outcome measures such as vital signs and the frequency of vasovagal episodes or fetal parameters such as Apgar score, birth weight, and frequency of fetal distress are crude and too easily influenced by factors other than those directly related to phlebotomy. However, methods now exist to study both the maternal and fetoplacental circulatory beds in a noninvasive and reproducible fashion.

The collection, storage, and use of autologous blood during the third trimester of pregnancy have been described since 1969.[1] Predonation and use of autologous blood is thought by some to be underused in the general population scheduled for elective surgery.[2] Although autologous blood donation appears to be gaining in overall popularity,[3] its utility in the pregnant population at risk for excessive obstetric blood loss remains under considerable debate.[4-6] Analysis of older case reports[1, 7, 8] and more recent published series[9-12] of autologous blood donation and administration during pregnancy appears to confirm the overall clinical safety of this blood banking technique. However, few if any cases have been studied in sufficient detail to permit conclusions about hemodynamic consequences of autologous donation other than maternal and fetal pulse and maternal blood pressure.

We report the results of a prospective study designed to characterize in some detail the maternal and fetal hemodynamic effects of the loss of 1 U of whole blood obtained for autologous use during the third trimester of pregnancy. These hemodynamic effects are compared with those observed in an age-matched cohort of nonpregnant female volunteer blood donors.

Material and methods

Study population. Sixteen pregnant women from the University of Washington and Harborview Medical Center antepartum clinics or patients referred by community obstetricians for this study were enrolled. In addition, we recruited 16 nonpregnant women from volunteers at the University of Washington women's clinics and at Puget Sound Blood Center blood drives. All study subjects met the following enrollment criteria: (1) reproductive age (18 to 40 years) with regular menses; (2) no history of vascular, renal, endocrine, or hematologic disease; (3) no vasoactive medication (including oral contraceptive) use or substance (including tobacco) abuse; (4) prephlebotomy fingerstick hemat-

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Table I. Hemodynamic data*

	Mean arterial pressure	*Cardiac output*	*Total peripheral resistance*	*Heart rate*	*Stroke volume*	*Fetal umbilical systolic/diastolic ratio*
Before phlebotomy—supine						
Control	84.26 ± 7.12	6.27 ± 1.57	1149 ± 336	66.6 ± 8.7	94.16 ± 21.82	—
Pregnant	76.71 ± 7.92	8.41 ± 2.05	821 ± 205	83.8 ± 10.7	95.79 ± 27.64	2.57 ± 0.51
Before phlebotomy—standing						
Control	89.41 ± 5.79	4.14 ± 1.29	1953 ± 616	77.6 ± 9.8	53.93 ± 17.32	—
Pregnant	85.84 ± 7.46	6.22 ± 1.29	1157 ± 294	95.0 ± 11.5	65.17 ± 11.70	2.42 ± 0.53
After phlebotomy—supine						
Control	80.59 ± 8.51	5.86 ± 1.42	1178 ± 388	65.1 ± 6.6	90.03 ± 22.64	—
Pregnant	78.73 ± 9.95	7.70 ± 1.97	898 ± 261	80.9 ± 6.9	93.96 ± 24.42	2.63 ± 0.84
After phlebotomy—standing						
Control	87.97 ± 9.23	3.99 ± 1.07	1894 ± 598	90.3 ± 14.0	44.86 ± 12.86	—
Pregnant	84.56 ± 7.74	5.95 ± 1.29	1181 ± 262	97.9 ± 9.6	59.61 ± 13.00	2.28 ± 0.27

*Expressed as mean ± 1 SD.

ocrit ≥34%; (5) American Association of Blood Banks standards for autologous or homologous blood donation met[13]; and (6) informed consent obtainable. All pregnant subjects met the following additional criteria: (1) singleton pregnancy at 24 to 36 weeks' gestation; (2) normotensive at first prenatal visit and at enrollment; (3) no premature labor or abnormal placentation; and (4) normal fetal growth with no fetal anomalies.

Instrumentation. The maternal and volunteer donor circulation were studied by A- and B-mode ultrasonography. With the UltraCOM cardiac output monitor and the methods described by Easterling et al.[14] ultrasonographic measurements of the aortic root diameter were obtained; these measurements were used to calculate the aortic cross-sectional area. A time-velocity profile of blood flow in the ascending aorta was generated with continuous wave Doppler ultrasonography through a suprasternal window. Cardiac stroke volume was calculated by $SV = CSA \int v\,(t)\,dt$, where SV is stroke volume, CSA is aortic cross-sectional area, v is velocity, and t is ejection time. Maternal cardiac output was then calculated by CO = SV (HR, where CO is cardiac output and HR is heart rate. Blood pressure was measured every 5 minutes with an Accutorr (Datascope, Paramus, N.J.) automated cuff on the arm contralateral to the phlebotomy site. Systemic vascular resistance was calculated as TPR = 80 (MAP)/CO, where TPR is total peripheral vascular resistance and MAP is mean arterial pressure. All UltraCOM measurements were performed by an experienced research nurse whose proficiency in this method had previously been validated by close correlation with invasively obtained cardiac outputs in both pregnant and nonpregnant patients.[15]

The fetal umbilical artery flow velocity waveform was obtained with a Medasonics Vasculab D-10 continuous-wave Doppler with an insonating frequency of 4 MHz. During a period of fetal inactivity and apnea the umbilical artery waveform was identified and printed. The maximum systolic and minimum diastolic flow velocities were measured by hand, the systolic/diastolic ratio was calculated. Two separate measurements over a minimum of five waveforms were averaged. Fetal heart rate (FHR) and uterine activity were continuously monitored with a third-generation Hewlett Packard electronic fetal monitor (Hewlett Packard, Germany) with an external Doppler probe for FHR and a tocodynamometer strain gauge for uterine activity.

Study protocol. The initial fingerstick for the screening hematocrit and the subsequent phlebotomy for 1 U of whole blood were carried out by a certified phlebotomist. Collection, identification, transport, processing, and storage of the autologous units from the pregnant study group and the homologous units of blood from the control group were carried out in strict compliance with American Association of Blood Banks guidelines.

After the predonation history and informed consent were obtained, the subject was settled into semi-Fowler recumbent position. In pregnant subjects the external fetal monitors were applied, and the screening hematocrit was spun. Blood pressure, cardiac output, and fetal Doppler measurements were concurrently obtained with the woman in the supine position. Thereafter, the subjects were asked to rise to the standing position, fetal monitors were adjusted, and the vital signs were allowed to equilibrate for 5 minutes, at which time hemodynamic measurements were repeated. The subject was then returned to a recumbent position, and phlebotomy for 450 ml of whole blood was carried out. After a 10-minute equilibration period measurements were repeated, the subject was gradually returned to the standing position, and a final set of hemodynamic parameters was obtained.

Data analysis. The data were analyzed with a microcomputer statistical software package (StatView 512, Version 1.1, Abacus Concepts Inc., Brain Power, Cal-

Table II. Results of one way analysis of variance

Dependent variables	*Independent variables*					
	Pregnancy		*Orthostasis*		*Phlebotomy*	
	F statistic	*Significance*	*F statistic*	*Significance*	*F statistic*	*Significance*
Mean arterial pressure	8.43	$p < 0.01$	23.74	$p < 0.01$	0.60	NS
Cardiac output	51.18	$p < 0.01$	50.18	$p < 0.01$	1.40	NS
Total peripheral resistance	56.56	$p < 0.01$	57.84	$p < 0.01$	0.07	NS
Heart rate	67.65	$p < 0.01$	82.99	$p < 0.01$	2.47	NS
Stroke volume	5.11	$p < 0.05$	115.96	$p < 0.01$	2.18	NS
Fetal umbilical systolic/diastolic ratio	—	—	0.09	NS	3.11	NS

NS, Not significant.

abasas, Calif.) by means of a one- or two-tailed paired Student t test for comparisons of hemodynamic variables. The relative impact of position, pregnancy, and phlebotomy was analyzed by one-way factorial analysis of variance, with the hemodynamic parameters serving as the dependent variable and pregnancy, maternal position, and blood loss as the independent variables. A value of $p \leq 0.05$ was chosen to represent statistical significance.[16]

Human subjects. The study had University of Washington Institutional Review Board approval and was carried out in the Clinical Research Center of University Hospital. Informed consent for the study protocol and a standard consent for blood donation were obtained, and all subjects were made aware that the blood would be subject to screening for hepatitis and human immunodeficiency virus. Pregnant study subjects were also made aware that their autologous blood would be transfused only if clinical indications warranted it and that the blood, if unused, would not be added to the homologous donor pool. The study subjects were not financially compensated.

Results

A summary of the hemodynamic data is shown in Table I. As expected from the physiologic changes known to occur during pregnancy, pregnant subjects had significantly higher cardiac outputs ($p < 0.05$) and heart rates ($p < 0.05$) than did nonpregnant controls. Similarly, peripheral vascular resistance was significantly lower in the pregnant state ($p < 0.05$). The lower mean arterial pressure known to be associated with pregnancy ($p < 0.05$) was, however, only seen during recumbency before phlebotomy.

Orthostasis provoked significant changes in all maternal hemodynamic parameters in both the study and control groups before and after blood loss. Mean arterial pressure, peripheral vascular resistance, and heart rate increased, whereas cardiac output and stroke volume decreased ($p < 0.01$ for all parameters, one-tailed Student t test).

We analyzed the relative effects of pregnancy, orthostasis, and blood loss on the hemodynamic parameters we studied with one-way factorial analysis variance (Table II). When the effects of pregnancy ($p < 0.01$) and orthostatic stress ($p < 0.01$) were taken into account, the impact of a 450 ml blood loss became negligible ($p > 0.05$).

In both the pregnant and control group, the effect of phlebotomy on the subjects' responses to orthostatic stress was insignificant for cardiac output, peripheral vascular resistance, mean arterial pressure, and stroke volume ($p > 0.05$, paired Student two-tailed t test). Only the heart rate response to orthostasis was significantly different, in that a slightly more pronounced heart rate increase was observed after phlebotomy in both pregnant ($+5.82 \pm 2.2$ beats/min, $p < 0.05$) and control subjects ($+14.2 \pm 6.3$ beats/min, $p < 0.01$). In pregnant subjects, however, the magnitude of this change was significantly less pronounced than in controls ($p < 0.05$).

Neither phlebotomy nor orthostasis had significant effects on the fetal umbilical systolic/diastolic ratio ($p > 0.05$, analysis of variance) (Table II). No abnormal FHR patterns or excessive uterine activity were observed.

Two subjects (one pregnant and one control) had vasovagal episodes on arising after phlebotomy. Both events were self-limited and responded readily to additional recumbency. The FHR tracing remained normal throughout the episode.

Comment

The physiologic impact of acute blood loss in the context of blood donation has not previously been reported for either pregnant or nonpregnant individuals. The present study demonstrates that measurement of blood pressure and heart rate are only crude tools in

the evaluation of physiologic changes that may be present during periods of hemodynamic stress. Although there were profound and significant differences between pregnant and control groups in almost all hemodynamic parameters both in the supine and standing positions before and after blood loss, the mean arterial pressure reflected these differences only during recumbency before phlebotomy. This points to the need for a more detailed characterization of the hemodynamic effects of blood donation in both pregnant and nonpregnant subjects.

The noninvasive methods we used to study the donor circulation were first described by Huntsman et al.[17] and have been extensively compared with thermodilution-generated cardiac output measurements in both nonpregnant adults[18, 19] and pregnant women.[14] The correlation between thermodilution and Doppler ultrasonographic cardiac output determination in pregnancy is tight ($r = 0.91$).[15]

These noninvasive methods have previously been used to study the maternal hemodynamic effects of postural change in normal pregnancies.[20] In a study of 15 nonpregnant reproductive aged women, 14 pregnant women in the first trimester, and 16 pregnant women in the third trimester Easterling et al.[20] demonstrated a mean fall in cardiac output of 1.7 to 1.8 L/min with change from the recumbent to the standing position. Systemic vascular resistance rose in each of the groups. The most marked rise was observed in the nonpregnant group, with the smallest rise occurring during the third trimester of pregnancy.

The current study again demonstrates the increase in peripheral vascular resistance and the decline in cardiac output with orthostatic stress. The loss of 1 U of blood had no significant effect on the relative magnitude of these orthostatic responses in either pregnant women or in control subjects. Except for a small increase in the heart rate response, there appears to be no significant hemodynamic (mean arterial pressure, total peripheral resistance or cardiac output) impact on the orthostatic responses to phlebotomy in either pregnant or nonpregnant subjects.

Although pregnant and nonpregnant individuals responded similarly to both orthostatic stress and phlebotomy, some quantitative differences were observed. The increase in total peripheral vascular resistance in response to orthostatic stress both before and after phlebotomy was more pronounced in the control group. The same was true for the enhanced tachycardia seen in response to orthostatic stress after phlebotomy. These data and the absence of significant symptoms during and after blood donation in the pregnant study group support the notion that the maternal vasculature in healthy pregnancy, presumably because of its decreased reactivity to adrenergic stresses and volume loading, is relatively protected from hemodynamic changes, such as increases in vascular resistance, that are observed in nonpregnant individuals and that could conceivably pose a hazard to the developing fetus.

We recognize that the umbilical systolic/diastolic ratio, which was used to study the potential hemodynamic consequences or orthostasis and phlebotomy on the fetal circulation, permits only very limited conclusions about acute changes occurring in the fetal circulation as a result of maternal hemodynamic interventions. However, others have described measurable changes in the umbilical flow velocity waveform after maternal afterload reduction from lumbar epidural analgesia,[21] and after maternal bed rest and medical therapy in hypertensive pregnancies.[22, 23] We thus consider the absence of any consistent increase in the umbilical artery systolic/diastolic ratio in response to orthostasis or phlebotomy to be reassuring. It is further encouraging that continuous electronic fetal monitoring failed to show any abnormalities of the FHR tracing or increases in maternal uterine activity.

We thus conclude that in uncomplicated pregnancies during the third trimester the quality of the maternal hemodynamic response to the loss of 1 U of blood is similar to the response of healthy nonpregnant women and that the magnitude of this hemodynamic response appears to be somewhat less pronounced than in nonpregnant individuals. Although we cannot suggest that pregnant women are the ideal blood donors, it appears that ordinary periods of orthostatic stress, to which all pregnant women are subjected, have significantly more hemodynamic consequences than does the loss of 1 U of blood. No adverse fetal consequences resulted from the maternal hemodynamic changes that were observed as a result of blood donation. Continuous electronic fetal monitoring may thus be unnecessary if autologous blood donation is planned in a healthy near-term pregnancy.

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First-trimester diagnosis of cystic hygroma—Course and outcome

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OBJECTIVE: We studied the outcomes of fetuses in whom cystic hygroma was diagnosed in the first trimester of pregnancy through the application of transvaginal ultrasonography.

STUDY DESIGN: In the period 1990 to 1991 22 fetuses with cystic hygroma were found. All fetuses had karyotyping and a complete ultrasonographic search for associated anomalies.

RESULTS: Aneuploidy was found in seven of 22 fetuses: four trisomy 21, two trisomy 18, and one translocation. Monosomy X was absent in this series. In 15 of 22 cases there was a normal karyotype. In 10 of 15 euploid fetuses the small nonseptated hygroma resolved spontaneously. In four of 15 euploid fetuses other malformations were detected with ultrasonography (i.e., polycystic kidneys, coarctation of the aorta, bladder outlet obstruction, and fetal hydrops).

CONCLUSION: Whenever a cystic hygroma is suspected in the antenatal period, even if of small size, a structured and detailed ultrasonographic examination and fetal karyotyping are recommended. (Am J Obstet Gynecol 1992;167:94-8.)

Key words: Cystic hygroma, nuchal structures, transvaginal ultrasonography, first-trimester diagnosis, aneuploidy

High-frequency ultrasonography, especially through transvaginal application, allows early detection of fetal anomalies. Diagnosis in the first trimester of pregnancy has been reported for several fetal defects (e.g., gastroschisis, omphalocele, spina bifida, and cystic hygroma).[1-3] Other anomalies such as omphalocele cannot always be definitely diagnosed in the first trimester of pregnancy. Protrusion of fetal intestines anterior to the abdominal wall may reflect normal physiologic herniation.[4]

Although cystic hygromas are commonly found in first trimester abortuses,[5] the incidence at birth is rare (1/6000).[3] The introduction of transvaginal ultrasonography has dramatically increased the frequency of detection of first trimester cystic hygromas. Association of cystic hygromas with other structural defects and chromosomal abnormalities has been reported, although, on the other hand, regression of the hygroma has been observed with a good neonatal outcome.[3, 6]

This report describes the outcome of 22 fetuses with cystic hygroma diagnosed in the first trimester of pregnancy.

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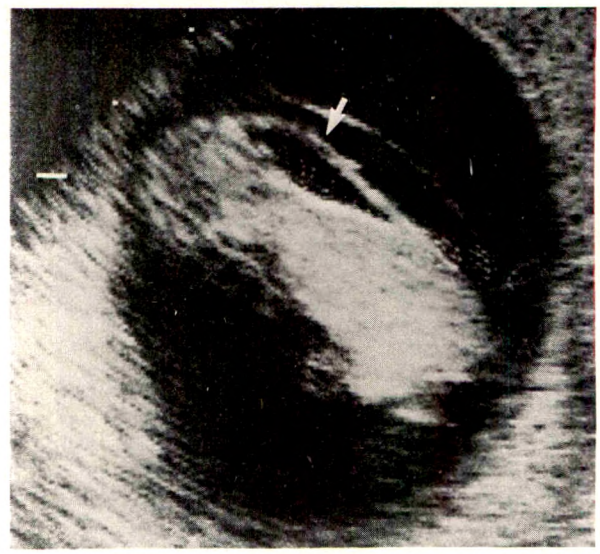


Fig. 1. Longitudinal view of small nonseptated hygroma *(arrows)* in fetus with normal karyotype.

Subjects and methods

Ultrasonographic scans were performed with a 5.0 or 7.5 MHz transvaginal sector scanner (Acuson 128, Mountain View, Calif.). In each case ultrasonographic examination was done for obstetric reasons such as pregnancy dating or vaginal blood loss. Cystic hygromas, which originated in the soft tissue with an intact skull and spinal column, were identified as single or multiloculated, fluid-filled cavities in the lateral or pos-

Table I. Outcome of fetuses with cystic hygromas

Gestational age	*Karyotype*	*Septation*	*Associated anomalies*	*Outcome*
10	46,XY	−	−	Normal
12 wk 2 days	46,XX	−	−	Normal
12 wk 3 days	46,XY	−	−	Normal
13	46,XY	−	−	Normal
13	46,XY	−	−	Normal
13	46,XX	−	−	Normal
13 wk 3 days	46,XY	−	−	Normal
13 wk 3 days	46,XY	−	−	Normal
14	46,XX	−	−	Normal
15 wk 2 days	46,XX	−	−	Normal
12 wk 3 days	46,XX	−	+*	Noonan's syndrome
10 wk 1 day	46,XY	−	+†	Termination of pregnancy
13 wk 3 days	46,XY	+	−	Termination of pregnancy
14 wk 3 days	46,XY	+	+‡	Termination of pregnancy
15 wk 2 days	46,XX	+	+§	Termination of pregnancy
10 wk 5 days	47,XY,+21	−	−	Termination of pregnancy
12 wk 5 days	47,XX,+21	+	−	Termination of pregnancy
13 wk 5 days	47,XX,+21	−	−	Termination of pregnancy
15 wk 5 days	47,XX,+21	+	−	Termination of pregnancy
12 wk 5 days	47,XY,+18	+	+‖	Termination of pregnancy
15 wk 5 days	47,XX,+18	−	+¶	Termination of pregnancy
12 wk 6 days	45,X,−15,+der(15)+t(Y;15)	−	−	Termination of pregnancy

*Cardiac anomaly.

†Polycystic kidneys.

‡Urethral stenosis with large bladder.

§Hydrops. This patient had a previous child with a large cystic hygroma; an autosomal recessive type of cystic hygroma may be considered.

‖Omphalocele and clubbed feet.

¶Omphalocele.

terior cervical region. They all measured >3 mm in diameter and were either septated or nonseptated.[7] Whenever a cystic hygroma was suspected, a careful search for associated malformations was performed.[8-10]

Karyotype analysis was available from all patients through either chorionic villus (four transcervical, 16 transabdominal) or amniotic fluid (two) sampling. Autopsy was performed in all cases of elective abortion. In cases of small nonseptated cystic hygromas with a normal karyotype the patients had regular weekly ultrasonographic controls until the anatomic finding had resolved. An ultrasonographic examination was performed to detect or exclude additional (cardiac) anomalies at 16 to 18, 22 to 24, and 31 to 32 weeks of pregnancy.

Results

The malformations were detected in all 22 cases between 10 and 16 weeks of gestation, as listed in Table I. The mean gestational age at detection was 13.1 weeks (SD ± 1.6 weeks). Chromosome analysis was available from all 22 cases; seven fetuses (32%) had an abnormal and 15 fetuses (68%) a normal karyotype. In 10 of the euploid cases (67%) the posterior nonseptated hygromas could no longer be detected with ultrasonography after 16 weeks of gestation, and no signs of a disturbance in lymphatic drainage or other nuchal malformations such as webbed neck could be detected after birth (Fig. 1). Two of these fetuses had a single umbilical artery. Both infants were born at term and did not show any morphologic abnormality. In four euploid fetuses associated malformations were detected. One fetus had a cardiac anomaly, consisting of a small atrial and ventricular septal defect and a coarctation of the aorta, detected in the third trimester of pregnancy and confirmed after delivery. In this case the hygroma was partially absorbed. The ultrasonographic appearance changed during pregnancy from a translucent structure to a thick echogenic nuchal fold. The child was born with a webbed neck. Noonan's syndrome was considered, although the cardiac anomaly originally described with this single gene disorder consists of pulmonary stenosis. One pregnancy with a euploid fetus in which the hygroma had resolved showed later on severe oligohydramnios. At 22 weeks polycystic kidneys and absence of bladder filling were detected. The malformations in the third fetus consisted of bladder outlet obstruction and deformation of the legs. In the fourth case there was a serious progressive septated hygroma in the neck and axillary region in association with fetal hydrops. One pregnancy with a euploid fetus was terminated on the patient's request.

Septation of the hygroma was found in six of 22

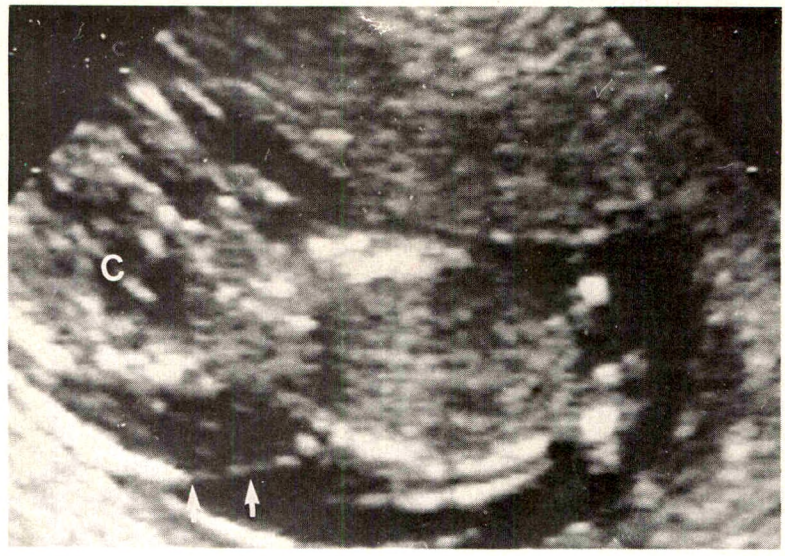


Fig. 2. Large sonolucent hygroma *(arrows)* in fetus with trisomy 18. *C*, Caput.

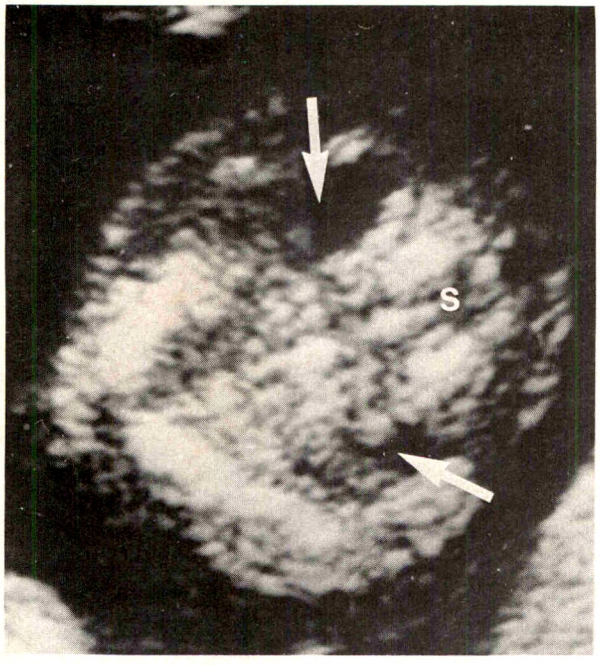


Fig. 3. Transverse scan through cervical spine *(S)*, with lateral localized translucent cysts *(arrows)* in fetus with trisomy 21.

fetuses, whereas 16 of 22 were nonseptated. Three fetuses with a septated hygroma had aneuploidy and two fetuses had a normal karyotype but demonstrated associated anomalies. One fetus with a septated hygroma was euploid and had no associated malformations. Of the 16 cases with a nonseptated hygroma four were aneuploid (one with associated anomalies), two had associated anomalies with a normal karyotype, and 10 fetuses were euploid and showed no other malformations.

In 21 of the 22 cases the hygroma was located posterior in the neck area (Fig. 2). In one case the malformation was lateral in the cervical region. Trisomy 21 was diagnosed (Fig. 3). All pregnancies with abnormal karyotypes (seven cases) were electively aborted as were four euploid pregnancies, three because of the associated anomalies and one because of serious mental instability in the mother. Autopsy results were available from all terminated pregnancies. The small nonseptated hygromas ultrasonographically detected in four of the seven aneuploid cases could not be identified by the pathologist at autopsy, probably due to lytic processes of the fetal material. The septated hygromas could all be identified by the pathologist at autopsy.

Comment

Fetal cystic hygromas are congenital malformations of the lymphatic system manifested as single or multiloculated fluid-filled cavities in the neck region. The posterior or lateral position and cystic appearance are characteristic.[11] The lymphatic vessels normally drain into two large sacs lateral to the jugular vein.[12] In case of a defect in the formation of the lymphatic vessels, the jugular sacs enlarge and lymph accumulates in tissues. The hygroma may regress and resolve if collateral routes develop or the jugular veins connect.[13, 14] Larger hygromas are often divided by a midline septum in the nuchal region. Extension to the fetal skull may be present. This feature has to be differentiated from other craniocervical masses such as encephalocele or meningomyelocele, teratoma, hemangiomas, and familial nuchal bleb.[15-17] Furthermore, one should be aware of diagnostic errors, i.e., a segment of the umbilical cord behind the neck, prominent fetal hair (later in pregnancy), an adjacent subchorionic cyst, or a part of the amnion not yet merged with the chorion in the first

trimester. Cystic structures in the neck region have been described in various sizes. Cullen et al.[3] refer to nuchal blebs if the size is <3 mm. In that case the cystic structure is always nonseptated and disappears before the eleventh week.

If a small cystic hygroma in the nuchal region has been detected there is also the possibility of a pseudomembrane,[18] especially when the suspected structure is not found in follow-up sonograms. Either the small cystic hygroma has absorbed or a dorsal specular reflection originating from the surface of the skin had been identified during the first examination.

Larger hygromas have often been described as caused by Turner's syndrome.[19] Besides monosomy X, which is often associated with generalized edema and coarctation of the aorta, several other chromosomal disorders have been reported, such as trisomy 21, 18, and 13.[20, 21] All these syndromes have a low recurrence risk. Other syndromes with a normal karyotype such as familial pterygium colli, Robert's syndrome, and Noonan's syndrome have been described with cystic hygroma.[11, 22, 23] The main features of Noonan's syndrome are very similar to monosomy X but are easily distinguished after birth facially, cytogenetically, and gonadally. Noonan's syndrome is often associated with hypertelorism, ptosis, dental malocclusion, lowset ears, broad nasal base, webbed neck, and low posterior hairline, and the affected children have normal ovaries 46,XX, or testis 46,XY. The associated congenital heart disease in Noonan's syndrome usually consists of pulmonary stenosis. This syndrome is caused by a single gene disorder and has a recurrence risk that may be much higher than in Turner's syndrome; it has a familial predilection and is probably transmitted in an autosomal dominant manner with incomplete penetrance. An extensive ultrasonographic examination is still the diagnostic choice in future pregnancies.[24]

In the current series monosomy X was not diagnosed. Several authors describe 50% association of Turner's syndrome with cystic hygroma. It always concerned large septated cystic hygromas, often in combination with fetal hydrops. Transvaginal application and modern high-resolution equipment makes it possible to visualize smaller cystic hygromas early in pregnancy, structures often not detectable with abdominal ultrasonography. More embyros and fetuses will be identified with a cystic hygroma in the first trimester of pregnancy than before. Early detection enlarges the group with these findings drastically. Cytogenetic analysis on villus material obtained from retained abortions in the first trimester revealed an abnormal chromosome constitution in 77%.[25] The relative proportion of chromosome abnormalities in retained abortions is higher for trisomies (61%) than for 45,XO (7.5%).[25] This eight-times-higher proportion may explain the absence of a monosomy X in our series.

Not all pregnant women have an ultrasonographic examination in the first or early second trimester of pregnancy. A cystic hygroma may have resolved if the sonogram is made in the second or third trimester. It is possible that some cases of Down syndrome obvious at birth could have been diagnosed early in pregnancy if the nuchal region had been inspected ultrasonographically in the first trimester. Hygromas can also be associated with trisomy 18 or 13, but the majority of these cases will be detected by ultrasonography either in the first trimester or later in pregnancy because of associated anomalies. In most of these cases the diagnosis can be suspected prenatally and confirmed by chromosome analysis.

In conclusion, the following recommendations can be made. A close look at the nuchal region should be part of every thorough investigation for congenital anomalies, especially in the first trimester. The finding—even if of limited size—has to be followed by a detailed ultrasonographic examination, and fetal karyotyping should be offered.[7] In case of euploidy and a resolving cystic hygroma, or if the aspect of the hygroma changes during pregnancy, a close look for cardiac anomalies is advisable.

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Baking powder pica mimicking preeclampsia

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We report a case of baking powder pica during pregnancy that was associated with maternal hypertension, hypokalemia, and elevated liver function tests. After discontinuation of baking powder ingestion and correction of electrolyte abnormalities, the blood pressure and the liver function tests normalized. (AM J OBSTET GYNECOL 1992;167:98-9.)

Key words: Pica, preeclampsia, pregnancy

Pica is an eating disorder manifested by the abnormal desire to ingest substances not generally considered for normal consumption. Previous investigations of the association of pica and toxemia of pregnancy noted a significant correlation with geophagia but not amylophagia.[1] Presented is a report of a woman who, while consuming large quantities of baking powder during her pregnancy, developed hypertension, hypokalemia, and elevated liver function tests.

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Case report

A 23-year-old black woman, gravida 3, para 2, was seen at 19.5 weeks' gestation with a 3-day history of headaches, dizziness, nausea, and vomiting. Blood pressure was 144/90 mm Hg with a first-trimester blood pressure of 110/50 mm Hg. Laboratory data included serum aspartate aminotransferase 144 U/L, lactic dehydrogenase 359 U/L, platelet count 226,000/mm^3, hematocrit 21.8%, and serum potassium 3.1 mEq/L. A urine drug screen was negative. During hospitalization, after transfusion of packed red blood cells for anemia and correction of hypokalemia, the blood pressure and liver function tests normalized. The patient was discharged home, but on a return clinic

visit at 33 weeks' gestation the blood pressure was 170/86 mm Hg and proteinuria (2+) was revealed. The patient was hospitalized and placed on a regimen of magnesium sulfate for seizure prophylaxis. Laboratory data included serum aspartate aminotransferase 308 U/L, lactic dehydrogenase 962 U/L with an increased LD_4 and LD_5 fraction, total bilirubin 1.7 mg/dl, serum potassium 1.4 mEq/L, platelet count 214,000/mm^3, and hematocrit 27.5%. Blood gas studies noted a pH of 7.49. A 24-hour urine collection noted a creatinine clearance of 177 mg/min with 991 mg protein. A human immunodeficiency virus antibody screen was negative.

A computed tomographic scan of the liver was performed, which showed a normal liver size and no focal hypodensities within the liver. Hounsfield unit measurements noted the same density as the spleen. A liver biopsy was performed, which demonstrates diffuse hepatocellular swelling accompanied by portal venous dilatation and low-grade acute and chronic nonspecific portal triaditis. There was no necrosis, cholestasis, or hemorrhage. This was not consistent with the pathologic findings of preeclampsia, acute fatty liver of pregnancy, or viral hepatitis.

Although previously denying pica, on further questioning the patient admitted to a 1½ year history of ingesting Calumet (General Foods, White Plains, N.Y.) baking powder in quantities up to one can (7 ounces) per day. The patient related that this was a family remedy for the relief of gas discomfort. Again during hospitalization, after correction of hypokalemia and discontinuation of baking powder ingestion, the blood pressure and liver function tests normalized.

The patient returned in active labor at 40 weeks' gestation, at which time she admitted to continued baking powder pica. Admission blood pressure was 138/102 mm Hg with a serum potassium level of 2.4 mEq/L and hematocrit of 23.3%. A primary cesarean section was performed for secondary arrest of dilatation with delivery of a 4235 gm male infant with Apgar scores of 8 and 9 at 1 and 5 minutes, respectively. The umbilical cord blood gas noted a pH of 7.63, P_{CO_2} 19 mm Hg, and P_{O_2} 27 mm Hg with a base excess of 1.7 mEq/L.

Comment

Pica is a persistent compulsive ingestion of substances not generally considered for normal consumption. Pica is more common in black women and those women with a positive childhood and family history of pica. The most common forms of pica include consumption of clay (geophagia) or starch (amylophagia) but may include ice, coal, or soap. Although the prevalence of pica among pregnant women in high-risk groups declined between the 1950s and the 1970s, Horner et al.[2] reported that pica now affects about one fifth of high-risk women. Nutritional assessment and counseling are indicated in those patients with pica.

Baking powder is composed of 30% sodium bicarbonate with cornstarch, sodium aluminum sulfate, calcium acid phosphate, and calcium sulfate. The main effect of ingestion and chronic abuse is a hypokalemic-hypochloremic metabolic alkalosis. Other metabolic abnormalities, including hypernatremia and hypocalcemia, are possible. Transient albuminuria, neuromuscular irritability including hyperreflexia, and elevations in blood urea nitrogen may occur with metabolic alkalosis. Ingestion of baking powder has been described to cause an increase in blood pressure, but liver dysfunction seems to be an unusual finding. Acute withdrawal from baking powder may cause a hypoaldosterone-like state.

Treatment of electrolyte abnormalities associated with baking powder pica should begin with the diagnosis. Correction of chloride and potassium deficit may allow for renal excretion of bicarbonate. If severe alkalemia persists, intravenous ammonium chloride in normal saline solution may be used.

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Presacral neurectomy for the treatment of pelvic pain associated with endometriosis: A controlled study

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OBJECTIVE: Our objective was to evaluate the efficacy of presacral neurectomy combined with conservative surgery for the treatment of pelvic pain associated with endometriosis.
STUDY DESIGN: In a randomized, controlled study performed in a tertiary institution 71 patients with moderate or severe endometriosis and midline dysmenorrhea were randomly assigned to conservative surgery alone (n = 36) or conservative surgery and presacral neurectomy (n = 35). Main outcome measures were relief of dysmenorrhea, pelvic pain, and deep dyspareunia after surgery according to a multidimensional and an analog pain scale.
RESULTS: Presacral neurectomy markedly reduced the midline component of menstrual pain, but no statistically significant differences were observed between the two groups in the frequency and severity of dysmenorrhea, pelvic pain, and dyspareunia in the long-term follow-up. After presacral neurectomy, constipation developed or worsened in 13 patients and urinary urgency occurred in three and a painless first stage of labor in two.
CONCLUSION: Presacral neurectomy should be combined with conservative surgery for endometriosis only in selected cases. (Am J Obstet Gynecol 1992;167:100-3.)

Key words: Endometriosis, presacral neurectomy, pelvic pain

Presacral neurectomy first described by Jaboulay[1] and Ruggi[2] in 1899, is widely used, in addition to removal of adhesions and ovarian and peritoneal endometriotic implants, in the treatment of pelvic pain associated with endometriosis. This surgical procedure is considered useful by many authors,[3-6] although it is not without intraoperative risks and side effects. Moreover, almost all studies completed so far have been retrospective and have evaluated pain without an adequate investigation of the symptom itself or without the use of a pain scale.[7] In a recent prospective study Tjaden et al.[8] observed that midline dysmenorrhea was abolished in 100% of patients undergoing presacral neurectomy in addition to conservative surgery for endometriosis. However, their series was small, and the reported benefit exclusively concerned the midline component of dysmenorrhea, whereas no favorable effect was seen in the other components.

We performed a prospective study of a larger series to evaluate the efficacy of presacral neurectomy in addition to conservative surgery in the relief of pelvic pain associated with moderate and severe endometriosis.

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Material and methods

We recruited 78 consecutive patients with laparotomic or laparoscopic diagnosis of endometriosis stage III or IV of the revised American Fertility Society classification[9] undergoing conservative surgery at the First Department of Obstetrics and Gynecology, University of Milan, from June 1986 to January 1990. All the women reported moderate or severe midline or midline plus lateral menstrual pelvic pain, associated in some with intermenstrual pain or deep dyspareunia. Thirty-four were infertile. None had previously undergone surgery for gynecologic disease or received medical treatment in the 6 months before the operation. We obtained informed consent from each patient and approval from the institution.

The women were assigned to conservative surgery alone (n = 40) or to conservative surgery and presacral neurectomy (n = 38) according to a randomization list. Clinical and demographic data of the patients are shown in Table I. The sample size was based on the assumption that dysmenorrhea would resolve in 50% of the women who did not undergo presacral neurectomy and in 80% to 90% of those who did. The number of patients included gave the study a power of 80% for a difference of 30% and a power of 90% for a difference of 40% at a level of statistical significance of 0.05.

Before surgery all patients underwent gastroenterologic, urologic, and orthopedic evaluation to exclude other causes of pelvic pain. In addition, they all completed a questionnaire that requested information on

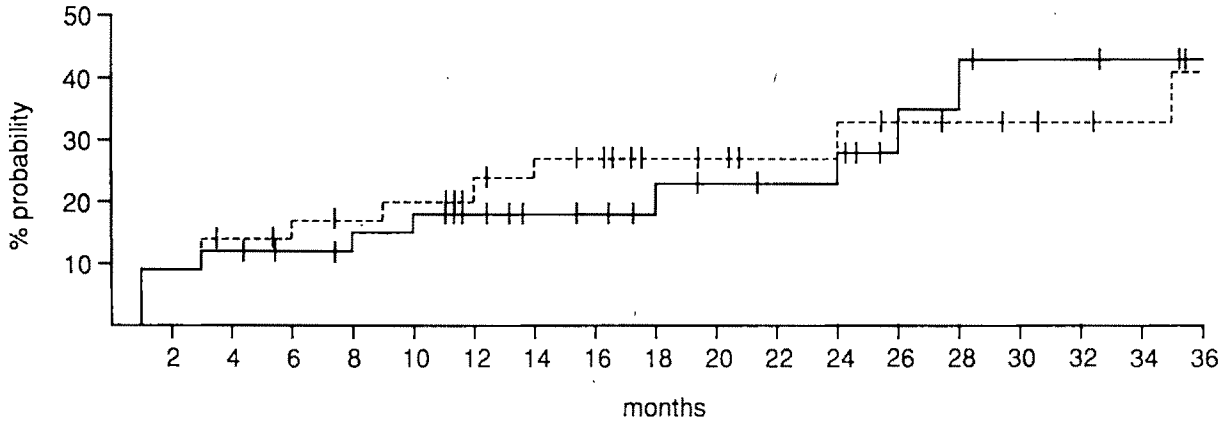


Fig. 1. Probability of recurrence of moderate or severe dysmenorrhea, according to linear pain scale, after presacral neurectomy in association with conservative surgery *(continuous line)* and after conservative surgery alone *(dotted line)*.

the presence, severity, and site of menstrual and pelvic pain and the presence and severity of deep dyspareunia. The severity of dysmenorrhea and pelvic pain was evaluated by means of a multidimensional scoring system, which is a modification of that proposed by Andersch and Milsom.[10] This system defines pain according to limitation of working ability (unaffected = 0, rarely affected = 1, moderately affected = 2, clearly inhibited = 3), coexistence of systemic symptoms (absent = 0, present = 1), and need for analgesics (no = 0, rarely = 1, regularly = 2, inefficacious = 3) and ranks their simple sum in three groups (0 to 3 = mild, 4 to 5 = moderate, 6 to 7 = severe). The women were also asked to specify the severity of dysmenorrhea and pelvic pain according to a 10-point linear pain scale in which 0 indicated the absence of pain, 1 to 5 mild pain, 6 to 7 moderate pain and 8 to 10 severe pain. The severity of deep dyspareunia was evaluated as described by Biberoglu and Behrman.[11] The women were asked to record monthly any pain symptoms after surgery. Every 6 months they underwent a clinical examination and the pain diary was checked. The follow-up was carried out in all patients for at least 1 year.

Conservative surgery was performed with the techniques described by Buttram and Reiter[12] and presacral neurectomy in accordance with Malinak's suggestions.[13] All adhesions and ovarian and peritoneal endometriotic implants were removed during surgery.

The pattern of dysmenorrhea in the postoperative follow-up was analyzed with the life-table method.[14] The patients with persistent or renewed, moderate, or severe pain were considered as treatment failures; the curve represents the cumulative probability rate of recurrence of moderate or severe dysmenorrhea.

We used the *t* test for unpaired data to compare the means and the χ^2 test to analyze the frequencies.

Table I. Characteristics of patients

	Conservative surgery (n = 36)	*Conservative surgery plus presacral neurectomy (n = 35)*
Age (yr, mean ± SD)	31.1 ± 3.6	32.5 ± 4.2
No. of parous women	5	6
No. of infertile women	19	15
Endometriosis stage		
III	26	23
IV	10	12

Results

Seven patients did not accept randomization, leaving 36 in the group for conservative surgery (group A) and 35 in that for conservative surgery plus presacral neurectomy (group B). According to the multidimensional and analog scales, dysmenorrhea before operation was moderate in 13 (36.2%) and eight (23%) of group A and in 10 (28.6%) and six (17.2%) of group B and severe in 23 (63.8%) and 28 (77%) of group A and in 25 (71.4%) and 29 (82.8%) of group B, respectively. In addition to midline dysmenorrhea, lateral menstrual pain was reported by 27 (75%) patients in group A and 24 (68.6%) in group B.

The mean time (±SD) taken to perform presacral neurectomy was 23 ± 8 minutes. No complications occurred during the operation, and no significant differences in postoperative morbidity (fever, days in hospital after surgery) were observed between the two groups. One patient underwent a second laparotomy 2 days after presacral neurectomy for a retroperitoneal presacral hematoma. Eleven subserous myomas (mean diameter 1.6 cm, range 0.6 to 3.5 cm) were enucleated or cauterized in eight patients of group A and 14 (mean diameter 1.7 cm, range 0.5 to 4 cm) in 10 patients of group B.

Figs. 1 and 2 show the pattern of dysmenorrhea during follow-up in the two groups, according to the two

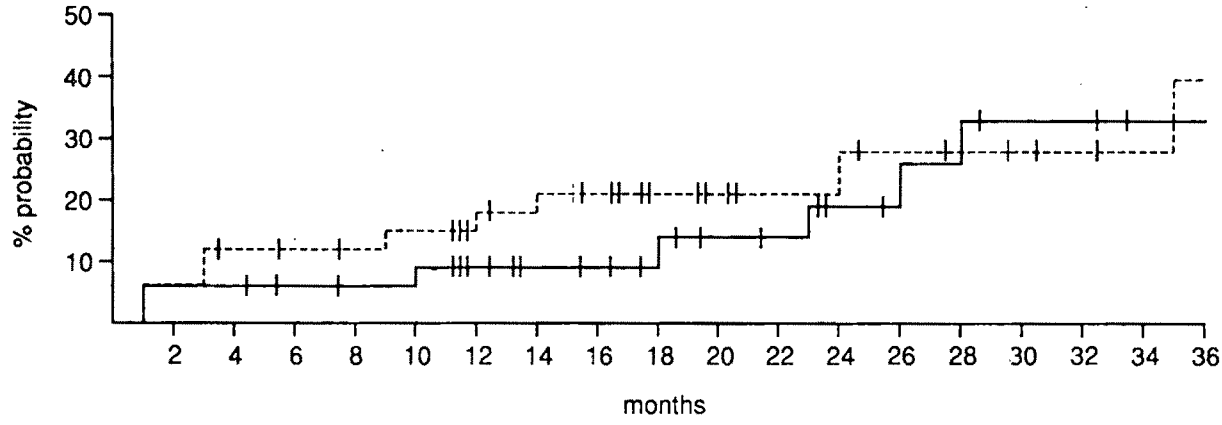


Fig. 2. Probability of recurrence of moderate or severe dysmenorrhea, according to multidimensional pain scale, after presacral neurectomy in asscciation with conservative surgery *(continuous line)* and after conservative surgery alone *(dotted line)*.

Table II. Frequency and severity of dysmenorrhea according to analog and multidimensional pain scale before and 12 months after surgery

	Conservative surgery (n = 36)		*Conservative surgery plus presacral neurectomy (n = 35)*	
Dysmenorrhea	*Before*	*After*	*Before*	*After*
Absent	0	21 (58%)	0	23 (66%)
Mild				
Analog	0	6 (17%)	0	5 (14%)
Multidimensional	0	8 (22%)	0	7 (20%)
Moderate				
Analog	8 (23%)	4 (11%)	6 (17%)	4 (11%)
Multidimensional	13 (36%)	4 (11%)	10 (29%)	3 (9%)
Severe				
Analog	28 (77%)	5 (14%)	29 (83%)	2 (6%)
Multidimensional	23 (64%)	3 (8%)	25 (71%)	2 (6%)

Table III. Frequency and severity of pelvic pain and deep dyspareunia before and 12 months after surgery

	Conservative surgery (n = 36)		*Conservative surgery plus presacral neurectomy (n = 35)*	
	Before	*After*	*Before*	*After*
Pelvic pain				
Absent	21 (58%)	31 (86%)	18 (51%)	32 (91%)
Mild				
Analog	4 (11%)	2 (6%)	3 (9%)	0
Multidimensional	6 (17%)	3 (8%)	6 (17%)	1 (3%)
Moderate				
Analog	6 (17%)	2 (6%)	8 (23%)	1 (3%)
Multidimensional	7 (19%)	1 (3%)	7 (20%)	1 (3%)
Severe				
Analog	5 (14%)	1 (3%)	6 (17%)	2 (6%)
Multidimensional	2 (6%)	1 (3%)	4 (11%)	1 (3%)
Deep dyspareunia				
Absent	18 (50%)	29 (81%)	16 (46%)	31 (89%)
Mild	5 (14%)	3 (8%)	4 (11%)	0
Moderate	7 (19%)	1 (3%)	6 (17%)	2 (6%)
Severe	6 (17%)	3 (8%)	9 (26%)	2 (6%)

pain scales. Table II shows the frequency and severity of dysmenorrhea 12 months after surgery. In this period dysmenorrhea recurred in 15 patients in group A and in 12 in group B. Recurrent menstrual pain had a midline component in 15 (100%) group A women and in eight (66.7%) group B subjects (median = 3.525, p = 0.06) and a lateral one in nine (60%) and 10 (83%) (p = not significant), respectively. The frequency and severity of intermenstrual pain and deep dyspareunia in the two groups before and 12 months after surgery are reported in Table III. Presacral neurectomy was associated with different side effects in the follow-up. Moderate or severe constipation developed or worsened in 13 patients. One had recurrent subocclusive crises due to the formation of fecal masses in the rectal ampulla. Three patients reported the appearance of urinary urgency. Two women who delivered after presacral neurectomy had a painless first stage of labor. One of them arrived at the hospital at 36 weeks of gestation, already in the second stage, with a breech presentation, and vaginal delivery was achieved without complications.

Comment

In our study the addition of presacral neurectomy to conservative surgery for moderate or severe endometriosis did not result in a greater reduction of pelvic pain than did conservative surgery alone. In fact, 1 year after the operation 80% of the patients who underwent presacral neurectomy did not have moderate or severe dysmenorrhea, compared with 75% of the controls. Af-

ter conservative surgery plus presacral neurectomy deep dyspareunia resolved or improved in 84% of patients and intermenstrual pain in 82%, compared with 67% and 80% of women who underwent conservative surgery alone.

Because of the relatively small size of our series, we cannot exclude the possibility that a small beneficial effect of presacral neurectomy on pain went unrecognized. However, our study had the power to demonstrate differences of ≥40%. Because presacral neurectomy may have important complications and side effects are frequent, an advantage of <40% should not, in our opinion, justify its routine performance.

After surgery the patients were informed whether they had undergone presacral neurectomy. It was not possible to make our study a blind one for organizational and medicolegal reasons. Knowledge of the surgical procedure performed could have influenced the patients' perception of pelvic pain. However, it has been demonstrated that in dysmenorrhea the placebo effect disappears within 3 months.[15]

In our analysis of the pattern of dysmenorrhea we used the life-table method to correct for variations in follow-up and the occurrence of pregnancy. The pattern of recurrence of dysmenorrhea was very similar in the two treatment groups.

Midline recurrent menstrual pain was reported by eight women (23%) who underwent presacral neurectomy and 15 (42%) who did not. Although the difference reaches the limit of statistical significance ($p = 0.06$), these data indicate that after presacral neurectomy recurrent dysmenorrhea could also have a midline component.

To date few clinical studies have evaluated the efficacy of conservative laparotomic surgery for endometriosis specifically in terms of pain relief.[7] Some retrospective nonrandomized studies have demonstrated that combining presacral neurectomy with conservative surgery offers a better resolution of dysmenorrhea and pelvic pain than simple excision of the endometriotic lesions.[3-6] However, in most reports the sample size was limited and the extent of endometriosis poorly defined, other pelvic diseases were often included, the length of follow-up was variable, and a pain scale was almost never used prospectively.

Recently Tjaden et al.[8] performed a randomized study to evaluate the efficacy of conservative surgery with and without presacral neurectomy in patients with moderate or severe endometriosis and chronic pelvic pain. They found that midline menstrual pain was completely abolished in 100% of the four patients who underwent presacral neurectomy with respect to the 0% of the four who did not. Similar findings were also observed in the 18 patients who refused randomization. However, the resolution of deep dyspareunia and acyclic pain was not greater in the cases versus the controls.

We conclude that there is still insufficient evidence showing that presacral neurectomy is indicated in the treatment of pelvic pain in all women undergoing conservative surgery for endometriosis. Endometriosis rarely provokes exclusively midline pain. Consequently, because presacral neurectomy markedly reduces only the midline component of dysmenorrhea, overall it cannot modify the subjective and social impact of the symptom. Thus presacral neurectomy for endometriosis should be performed only by skillful surgeons after a careful selection of the patients with mainly midline pain. Another probably useful criterion to determine the expectation of pain relief could be selective hypogastric nerve block performed before the operation.[16]

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Self-administered home cervicovaginal lavage: A novel tool for the clinical-epidemiologic investigation of genital human papillomavirus infections

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OBJECTIVE: Our objective was to evaluate a self-administered cervicovaginal irrigation apparatus (MY-PAP, Medtech, Bohemia, N.Y.) for the detection of cervicovaginal human papillomavirus infection.
STUDY DESIGN: Twenty-five women referred to a colposcopy clinic because of a recent abnormal Papanicolaou smear were studied. Human papillomavirus infection test results detected by Southern blot hybridization and polymerase chain reaction from physician-administered and MY-PAP self-administered cervicovaginal lavage were paired for comparison.
RESULTS: Seventeen (68%) patients returned home samples by mail a mean of 13 days after the clinic visit. All clinic samples and 16 of 17 (94%) home samples yielded adequate deoxyribonucleic acid for human papillomavirus infection analyses. Human papillomavirus infection was detected by polymerase chain reaction in 14 of 16 (88%) samples with complete concordance of viral detection in paired samples from the clinic and home. Southern blot detected human papillomavirus infection in 13 of 25 clinic samples (52%) and 7 of 16 home samples (44%), with concordance in 12 of 16 paired samples (75%). Specific viral type was the same in all positive paired samples (6/6). Concordance was high when the initial sample had a strong (2+ to 4+) human papillomavirus infection signal (5/6, 83%) or a negative result (5/6) or when a dysplastic (cervical intraepithelial neoplasia grade 1 to 3) lesion (9/11, 82%) was seen on cervical biopsy.
CONCLUSION: Self-administered lavage represents an extremely promising technique for obtaining cervicovaginal lavage samples for human papillomavirus infection analyses. (Am J Obstet Gynecol 1992;167:104-7.)

Key words: Cervicovaginal lavage, human papillomavirus infection, polymerase chain reaction, Southern blot

Human papillomavirus (HPV) infection has been implicated as an etiologic agent in the development of cervical neoplasia and cancer.[1] However, diagnosis of HPV infection has been hampered by the inability to culture the virus and the lack of reliable serologic tests. Diagnosis has required the use of molecular biologic techniques that identify the viral genome in cellular material from the lower genital tract.

Collection of samples for HPV analysis has been accomplished by cervical biopsy, scrape, swab, brush, or cervicovaginal lavage. Of the noninvasive methods, cervicovaginal lavage has proved superior in obtaining adequate samples for Southern blot analysis.[2, 3] However, significant limitations continue to impair the screening of large populations, including the availability of trained medical personnel, time requirements, patient inconvenience, or noncompliance, expense, and inability to reach medically underserved populations.

The recent development of MY-PAP (Medtech, Bohemia, N.Y.), a self-administered cervicovaginal irrigation device designed to obtain cellular material for cervical cytologic studies, offers promise for studies of genital HPV infection and cervical cancer screening. This study was undertaken to compare the results of HPV detection in cervicovaginal lavage samples collected in the clinic with those obtained at home by the patient using MY-PAP. Results of paired clinic and home samples were compared, thereby allowing each patient to serve as her own control.

Material and methods

Patient protocol. Twenty-five new patients from the colposcopy clinic of Bronx Municipal Hospital gave in-

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formed consent and were enrolled in this Institutional Review Board–approved study. All subjects had recent abnormal Papanicolaou smears. Gynecologic examination included Papanicolaou smear, cervicovaginal lavage, colposcopy, and directed cervical biopsy.

Patients were requested to obtain a self-administered cervicovaginal lavage sample with the MY-PAP kit approximately 10 days after the colposcopic examination. MY-PAP is designed and used like a douche. The device contains 21 ml of a physiologic saline solution in a small plastic bellows attached to a collection cup and a douchelike polypropylene tip with multiple openings designed to spray the irrigation solution outward in an omnidirectional pattern. After irrigation, release of the bellows apparatus causes the saline solution to be reaspirated through the tip, into the collection cup and bellows. Patients were provided with an ethanol-carbowax preservative and a preaddressed, postage-paid mail container to return the sample. No attempts were made to contact participants who failed to return the sample.

Cytology and histopathology. Cytologic slides were stained with the Papanicolaou stain, examined by a cytotechnologist, and reviewed by an experienced pathologist. Cervical cytologic results were reported with the Bethesda classification.[4] Biopsy specimens were formalin fixed, paraffin embedded, and stained with hematoxylin and eosin. Results were classified with the World Health Organization criteria for mild dysplasia (koilocytotic atypia, grade 1 cervical intraepithelial neoplasia), moderate dysplasia (grade 2 cervical intraepithelial neoplasia), or severe dysplasia (grade 3 cervical intraepithelial neoplasia–carcinoma in situ).[5]

Detection of HPV deoxyribonucleic acid (DNA). DNA was isolated from cellular material by proteinase K–sodium dodecyl sulfate digestion, followed by phenol-chloroform extraction and ethanol precipitation. Polymerase chain reaction was performed with the Manos L1 consensus primers.[6] Simultaneous amplification of HPV and a 110 bp cellular β-globin DNA fragment,[7] which served as an internal control of amplification efficacy, were performed as described by Bauer et al.[8] Polymerase chain reaction products were separated by electrophoresis in 3% NuSieve and 0.5% SeaKem agarose (FMC, Rockland, Me.) and transferred to nylon filters. A digoxigenin-labeled generic HPV probe was generated by the amplification of HPV 11, 16, and 18 with internal nested primers[6] in reactions that contained 200 μmol/L deoxyadenosine triphosphate, 200 μmol/L deoxyguanosine triphosphate, 200 μmol/L deoxycytidine triphosphate, 130 μmol/L deoxythymidine triphosphate, and 70 μmol/L digoxigenin-11-deoxyuridine triphosphate.[9] The probes were gel purified, denatured, and, together with a digoxigenin-11-deoxyuridine triphosphate end-labeled β-globin oligonucleotide probe,[6] added directly to filters that had been prehybridized in 5× saline sodium citrate, 0.02% sodium dodecyl sulfate, 0.5% blocking reagent (Boehringer Mannheim, Germany), and 0.1% *N*-lauryolsarcosine sodium salt. After overnight hybridization the filters were washed in 2× saline sodium citrate and 0.1% sodium dodecyl sulfate at 55° C, and the probe was detected by chemiluminescence with the Genius (Boehringer Mannheim) protocol.

Polymerase chain reaction products that were positive with the HPV generic probe were hybridized with type-specific oligonucleotide probes[8] for HPV 6/11, 16, 18, 31, 33, 35, 39, 45, 51, and 52. Aliquots of 3 μl of the initial polymerase chain reaction were denatured in 0.4 mol/L sodium hydroxide, and 25 mmol/L ethylenediaminetetraacetic acid, and applied to replicate filters with a 96-well dot blot apparatus. Filters were individually hybridized with the digoxigenin-11-deoxyuridine triphosphate end-labeled type-specific probes, and the blots were read blinded to other data. Samples that were positive by the generic probe mix but negative by all type-specific probes were considered to represent uncharacterized HPV types.

For Southern blot analyses approximately 5 to 10 μg DNA was digested by *Pst* I, separated by electrophoresis in an agarose gel, and transferred to a nylon filter. Low-stringency hybridization was performed with a mixed probe of phosphorus 32–labeled HPV 11, 16, and 18 DNA. Filters were washed under conditions of low stringency (TM − 40° C, 40° C below the DNA duplex melting temperature) and exposed to radiographic film at −70° C for 36 hours, followed by high-stringency (TM −10° C) washing and autoradiography for 10 days.

Southern blots were interpreted in a blinded manner. HPV typing was determined by the *Pst* I restriction enzyme pattern and the hybridization specificity.[10, 11] When distinct hybridization bands were present but the HPV type could not be determined, the virus was classified as "uncharacterized,"[12] and the approximate molecular weights of the DNA fragments were noted to allow later comparison of results from the clinic and home HPV samples. HPV hybridization signal strength was graded from weak to 4+ for each positive sample.[13]

Results

Seventeen (68%) patients returned samples they had obtained at home a mean of 13 (range 10 to 36) days after the clinic visit. There were no significant differences between the patients who returned the samples and those who did not with regard to age, initial HPV positivity, or cervical histopathologic findings (data not shown).

Cellular pellets from the home samples were somewhat bulkier and required longer digestion with proteinase K–sodium dodecyl sulfate than did samples ob-

Table I. Comparison of HPV test results on paired clinic and home cervicovaginal lavage samples

Subject No.	Cytology*	Biopsy	Clinic HPV testing			Home HPV testing		
			Southern		Polymerase chain reaction types	Southern		Polymerase chain reaction types
			Type	Signal		Type	Signal	
1	Negative	Not performed	0		16	0		16
2	High-grade lesion	Grade 1 cervical intraepithelial neoplasia, koilocytotic atypia	16	4+	6,11,16,18,31	16	3+	16,18,31
3	Atypical	Grade 1 cervical intraepithelial neoplasia, koilocytotic atypia	56	3+	U	56	1+	U
4	Atypical	Negative	35	1+	U	0		U
5	Low-grade lesion, koilocytotic atypia	Grades 1 to 2 cervical intraepithelial neoplasia, koilocytotic atypia	18	4+	16,18	18	4+	16,18
6	High-grade lesion	Grades 2-3 cervical intraepithelial neoplasia	U	1+	35,52	0		U
7	Low-grade lesion	Grade 2 cervical intraepithelial neoplasia	U	2+	U	U	3+	U
8	Negative	Endocervicitis	0		0	U	Weak+	0
9	High-grade lesion	Grade 3 cervical intraepithelial neoplasia	0		U	0		U
10	Low-grade lesion, koilocytotic atypia	Grade 2 cervical intraepithelial neoplasia	0		18,33	0		18,33
11	Negative	Grade 1 cervical intraepithelial neoplasia, koilocytotic atypia	0		0	0		0
12	High-grade lesion	Grade 2 cervical intraepithelial neoplasia	0		Technically inadequate	Technically inadequate		Technically inadequate
13	High-grade lesion	Grade 3 cervical intraepithelial neoplasia	18	2+	18	0		18
14	High-grade lesion	Grade 2 cervical intraepithelial neoplasia	18	2+	6,11,16,18	18	2+	6,11,16,18
15	Negative	Squamous metaplasia	U	1+	18,31,33	U	1+	18,31,33
16	High-grade lesion	Grade 1 cervical intraepithelial neoplasia, cervicitis	0		U	0		16
17	Technically inadequate	Not performed	0		U	0		U

U, Positive for uncharacterized HPV type; *0*, negative for HPV.

*All subjects had a history of abnormal Papanicolaou results. Papanicolaou results shown are from the last smear obtained before cervical biopsy and clinic cervicovaginal lavage. HPV was detected by Southern blot and polymerase chain reaction followed by type-specific probing. Home samples were obtained a mean of 13 (range 10 to 36) days after the clinic visit and biopsy. Southern blot signal strength was scored on a weak+ to 4+ scale as previously described.[13]

tained in the clinic. This may have been partially attributable to the carbowax in the MY-PAP preservative. Nevertheless, all clinic samples and 94% (16/17) of the home samples yielded DNA that was quantitatively and qualitatively adequate for Southern blot and polymerase chain reaction analyses. The one sample judged to be inadequate (Table I, patient 12) contained no visible cellular pellet, and attempts to amplify the β-globin fragment were unsuccessful, suggesting that the sample was devoid of amplifiable genomic DNA.

Polymerase chain reaction followed by hybridization with the generic probe mixture detected HPV in 88% (14/16) of the amplified samples from both clinic and home, demonstrating complete concordance as to the presence or absence of virus in paired samples (Table I). Although the results of type-specific probing after

polymerase chain reaction suggested that many patients were infected with multiple HPV types, the paired samples yielded the same results on all but three instances. One patient (No. 2) had positive results for HPV types 6/11, 16, 18, and 31 in the clinic and HPV types 16, 18, and 31 in the home sample. Another patient (No. 6) had HPV 35 and 52 detected in the clinic sample and an uncharacterized type detected in the home sample. The third patient (No. 16) had an uncharacterized HPV initially but was HPV 16 positive in the home sample.

HPV was detected by Southern blot in 52% (13/25) of the clinic samples and 44% (7/16) of the home samples ($p = 0.85$, χ^2). Seventy-five percent (12/16) of the paired samples (Table I) were concordant for the presence of HPV DNA. In addition, concordance was highest when the initial sample yielded a strong (2+ to 4+) HPV signal (83% or five of six) or a negative result (5/6). Concordant results were also observed more frequently in those patients who had dysplastic lesions (cervical intraepithelial neoplasia grades 1 to 3) on cervical biopsy (82% or 9/11) than those who had inflammatory changes or normal biopsy specimens (50%, 2/4). All positive paired samples (6/6) yielded the same viral type from both lavage specimens.

Comment

Seventeen of 25 patients returned home cervicovaginal lavage samples. The 68% compliance rate observed in this inner-city, municipal hospital population was judged to be quite acceptable, particularly since no reimbursement was offered for study participation and no attempt was made to contact those individuals who failed to return samples.

Samples obtained at home and preserved in ethanol-carbowax fixative were qualitatively and quantitatively adequate for Southern blot and polymerase chain reaction analyses. Polymerase chain reaction, the more sensitive diagnostic modality, demonstrated that the same viral types were present in both clinic and home samples. Indeed, the agreement in results on paired samples was striking. Furthermore, many patients tested positive for multiple viral types with polymerase chain reaction. Potentially, some of these patients may have been infected with uncharacterized viral types that cross hybridized with one or several type-specific probes.

The results of HPV detection by Southern blot demonstrated highest concordance in paired samples when the initial samples yielded either a strong signal or a negative result and in those patients who had dysplastic lesions on cervical biopsy. This suggests that discordant results occurred most often in patients with quantitatively less virus and in patients who lacked evidence of viral cytopathic effect or dysplasia on biopsy. Factors that may have contributed to discordant results include the elapsed time (averaging 2 weeks) between samples, day-to-day variation in viral shedding, or the intervening cervical biopsy, which may have decreased detectable virus.

We conclude the MY-PAP offers an easy, relatively inexpensive method of obtaining cervicovaginal lavage samples for HPV analysis. Patient compliance and acceptability of the device were high. This method offers great promise in clinical and epidemiologic studies of HPV infection and cervical neoplasia and may be particularly useful in studies that involve repetitive sampling or in the evaluation of medically underserved populations.

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Cold therapy in the management of postoperative cesarean section pain

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Sixty-two patients were randomized to receive either localized cold therapy to the cesarean section incision or routine postoperative care. Evaluation of the amount of analgesia requested, infection rate, and length of hospital stay did not show a significant difference between the two groups. There is no objective evidence to show that the use of cold therapy in postoperative cesarean section pain relief is beneficial. (Am J Obstet Gynecol 1992;167:108-9.)

Key words: Cold therapy, cesarean section, postoperative analgesia

Postoperative use of cold therapy is said to result in a quicker, less painful recovery from surgery and a decrease in the need for analgesic medications. It has been widely used after orthopedic procedures[1] but has not been studied in abdominal surgery. It acts by causing constriction of the cutaneous blood vessels, by diminishing bleeding and edema, and by raising the pain threshold and causing localized muscle relaxation.[2]

We carried out a prospective study to evaluate the efficacy of cold therapy in postcesarean wound analgesia. The Dual Temp localized cold therapy unit by Seabrook Medical Systems, Inc., was used. Sixty-two consecutive patients undergoing cesarean section at Temple University Hospital were enrolled for the study. Half were randomized to receive the cold therapy (group 1) whereas the other half had routine postoperative care (group 2). The disposable Dual Temp pad was applied to the operative incision after routine closure, wound cleansing, and application of a nonadhesive dressing. The unit, which circulates chilled water through its channels, has two self-sealing hoses that are left outside the bandages. The desired surface temperature is maintained by the central unit, thereby avoiding the problem of overcooling and damage to superficial structures. The temperature of the fluid is set at 43° F, and the unit is run continuously for 48 hours. However, the unit can be disconnected and reconnected for intermittent interruptions as required. Patients receiving epidural analgesics for long-term pain control postoperatively were excluded from the study.

Comparison of the two groups with regard to age, gravidity and parity, racial background, and prenatal care did not reveal a significant difference. Likewise there was no significant difference in the indications for cesarean section in the two groups. The majority of cesarean sections were performed with the patient under epidural anesthesia, with spinal or general anesthesia used in the rest of the cases. Postoperative parenteral analgesia was with meperidine hydrochloride and promethazine hydrochloride, followed by oxycodone or acetaminophen with codeine when bowel function returned. No significant difference was noted between the two groups in relation to the amount and duration for which postoperative analgesia was required. Patients with group 1 required an average of

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Table I. Comparison of two patient groups

	Cold therapy (n = 31)	*Control (n = 31)*	*p*
Postoperative analgesia			
Parenteral (mean)	3.09 (range 0-8)	3.08 (range 1-7)	NS
Total oral (mean)	2.68 (range 0-9)	3.16 (range 1-5)	NS
Return of gastrointestinal function (mean No. days to passing flatus)	1.9 (range 1-6)	2.0 (range 1-5)	NS
Postpartum endomyometritis	11 (35.4%)	13 (41.9%)	NS
Length of hospital stay (mean days)	4.58 (range 3-7)	4.83 (range 3-8)	NS

NS, Not significant.

3.09 injections followed by 2.68 oral doses of analgesia as compared with patients in group 2, who required 3.08 injections followed by 3.16 doses of oral analgesia. One patient in group 1 did not require any analgesia postoperatively. There was also no significant difference between the two groups in relation to the time to return of bowel activity, postoperative infection rate, and length of hospital stay (Table I).

This preliminary study fails to show a significant difference in requirement for postoperative analgesia or postoperative recovery when cold therapy is used in cesarean section patients. Experience in orthopedic surgery has shown cold therapy to be an effective means of reducing postoperative pain, especially in knee surgery and hip replacement, but this study failed to show similar outcome in the treatment of postoperative pain in abdominal surgery. It has been suggested that direct application of the cooling pad over the incision, as opposed to applying it over the surgical dressing, might enhance the physiologic effect, and this might warrant further study. In summary, it should be noted that at the present there is no objective evidence to show that the use of cold therapy in postoperative cesarean section pain relief is beneficial.

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Cyclooxygenase products do not modulate angiotensin II–induced contractions of human chorionic plate arteries

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OBJECTIVE: This study sought to determine whether cyclooxygenase products modulate angiotensin II–induced contractions of human placental arteries.

STUDY DESIGN: Ring segments of chorionic plate arteries (3 to 5 mm inner diameter) were obtained after normal vaginal deliveries. Active force n response to 10^{-6} mol/L angiotensin II or 10^{-4} mol/L arachidonic acid was measured in the presence or absence of 10^{-6} mol/L indomethacin to inhibit cyclooxygenase. The response of K^+ contracted arteries to the stable prostacyclin analog iloprost was also tested to determine whether prostacyclin relaxes these vessels. Statistical differences were assessed by analysis of variance, Wilcoxon sign rank test, or Student *t* test with Bonferroni correction for multiple comparisons.

RESULTS: Successive applications of angiotensin II produced significantly less contraction than the original dose (angiotensin II tachyphylaxis). Angiotensin II tachyphylaxis was unaffected by indomethacin. Arachidonic acid caused an indomethacin-sensitive contraction and iloprost failed to relax the arteries.

CONCLUSIONS: These results suggest that cyclooxygenase products do not mediate angiotensin II tachyphylaxis in these vessels. (AM J OBSTET GYNECOL 1992;167:110-4.)

Key words: Angiotension tachyphylaxis, prostaglandins, pregnancy-induced hypertension, prostacyclin, preeclampsia

Eicosanoids and vasoactive peptides have an important role in controlling blood flow in a number of different tissues, including the umbilicoplacental circulation.[1-5] These compounds appear to interact in many organs to regulate arterial tone, tissue blood flow, and vascular resistance. For example, vasodilator products of the cyclooxygenase pathway have been proposed to modulate angiotensin II–induced contractions in some peripheral blood vessels[5-7] (but not others[8-9]).

Alterations in the interactions between eicosanoids and other vasoactive stimuli may contribute to morbidity and mortality in some pathophysiologic states. For example, Minuz et al.[10] reported that the excretion of prostaglandin and thromboxane metabolites was altered in pregnancy–induced hypertension and concluded that an imbalance between constrictor and dilator influences in pregnancy-induced hypertension might produce vasoconstriction and potentiate the hypertensive effects of catecholamines and angiotensin II. Other authors[11, 12] have reported that vessels of preeclamptic individuals exhibit an enhanced responsiveness to angiotensin II and a diminished production of prostacyclin (PGI_2), which may be responsible for reduced blood flow and defective fetal nutrition[11] and could lead to the clinical manifestations of preeclampsia.[12]

To evaluate the potential role of altered angiotensin II responsiveness and reduced production of PGI_2 (or other cyclooxygenase products) in preeclampsia, it is essential to determine if these blood flow controllers interact in the normal placental vasculature. In the current study, we performed a series of experiments to test the hypothesis that the release of vasodilator products of the cyclooxygenase pathway modulates angiotensin II–induced contractions of the chorionic plate arteries on the fetal side of the human placenta.

Material and methods

General. The present experiments on placental vessels were conducted with the approval of the Human Research Review Committee of the Medical College of Wisconsin. Medium size chorionic plate arteries (3 to 5 mm inner diameter) were carefully dissected from human placentas obtained soon after normal vaginal

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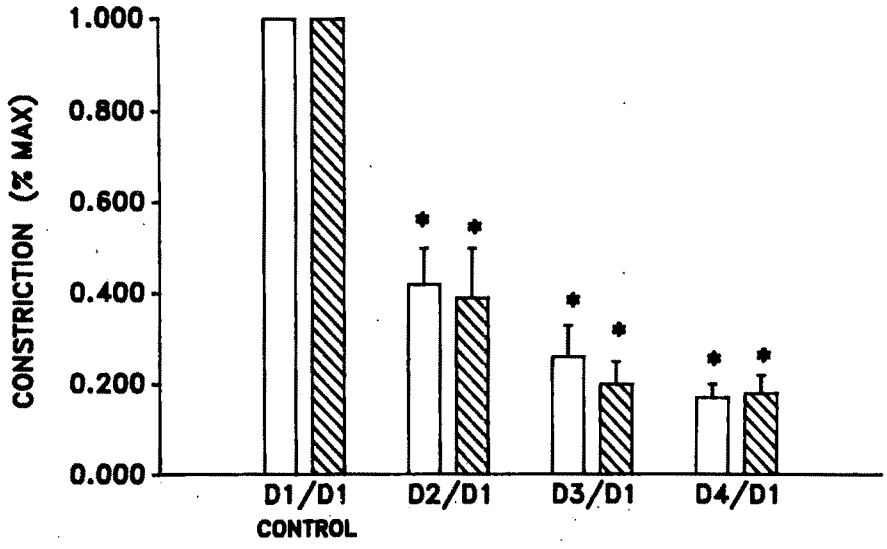


Fig. 1. Tachyphylaxis of human placental arteries to angiotensin II (10^{-6} mol/L) in presence of 10^{-6} mol/L indomethacin *(cross-hatched bars)* or vehicle *(open bars)*. Data are expressed as ratio of contractile response of vessel to given dose of angiotensin II *(D1, D2, D3, D4)* divided by the response to the first dose of angiotensin II *(D1)*. Successive applications of angiotensin II produced tachyphylaxis in both vehicle and indomethacin-treated rings. Data expressed as mean ± SEM for six arteries in each group. *Asterisk*, Significant decrease ($p < 0.05$) from initial response to angiotensin II in specific group. See text for details.

deliveries. Placental vessels were cut into 3 mm rings, mounted in 25 ml jacketed tissue baths, and maintained in physiologic salt solution containing 119 mmol/L sodium chloride, 4.6 mmol/L potassium chloride, 1.5 mmol/L magnesium chloride, 1.6 mmol/L calcium chloride dihydrate, 15 mmol/L sodium bicarbonate, 1.2 mmol/L sodium phosphate, and 11 mmol/L glucose. The vessel rings were maintained at 37° C and gassed with a 93% oxygen and 7% carbon dioxide gas mixture. Vessels were maintained at 3.0*g* passive tension and allowed to equilibrate for 1 hour before any experimental protocols. Changes in contractile force in the vessel were measured with Grass model FT-03 isometric force transducers connected to a Grass model 7 polygraph (Grass Instrument Co., Quincy, Mass.) or a CODAS electronic data acquisition system (Dataq Instruments, Inc., Akron, Ohio).

Specific protocols. Studies of tachyphylaxis to angiotensin II were performed by incubating the rings with indomethacin (10^{-6} mmol/L) or vehicle ($n = 6$ for each) for 30 minutes followed by four successive applications of 10^{-6} mol/L angiotensin II. The successive applications were separated by 30-minute washout periods. After the initial incubation period, indomethacin (10^{-6} mol/L) or its vehicle was present in the tissue bath throughout the experiment. Data were expressed as the ratio of active force in response to a given dose of angiotensin II divided by the response to the first dose of angiotensin II.

The effects of exogenous arachidonic acid (10^{-4} mol/L) were also tested on unstimulated arterial rings after 30 minutes of incubation with indomethacin (10^{-6} mol/L) or its vehicle. In this series of experiments, arachidonic acid (sodium salt, Sigma Chemical Co., St. Louis) was dissolved in ethanol to produce a 0.1 mol/L stock solution. Aliquots of the arachidonic acid stock solution were then added to the tissue bath to achieve a final concentration of 10^{-4} mol/L.

In another series of experiments, the responses of the vessels to PGI_2 were tested by adding the stable PGI_2 analog iloprost to rings that were precontracted with 40 mmol/L K^+. To determine whether PGI_2 had any dilator action on the vessels, the active force in K^+-contracted vessels treated with iloprost was compared with that in control rings that had been exposed to 40 mmol/L K^+ for an equivalent time without iloprost (K^+ time controls) and K^+-contracted rings that were treated with the nitrovasodilator sodium nitroprusside (10^{-5} mol/L).

Statistical procedures. Differences between successive angiotensin II responses in the presence and absence of indomethacin or its vehicle were assessed by comparing the absolute force developed in response with each dose of angiotensin II. The presence of significant differences was detected by analysis of variance, and differences between specific group means were assessed with a multiple range test. Responses to exogenous arachidonic acid in the presence and absence of indomethacin were compared with the Wilcoxon sign rank test. Differences in active force in iloprost-treated vessels versus K^+ time controls and nitroprusside-treated vessels were compared with a

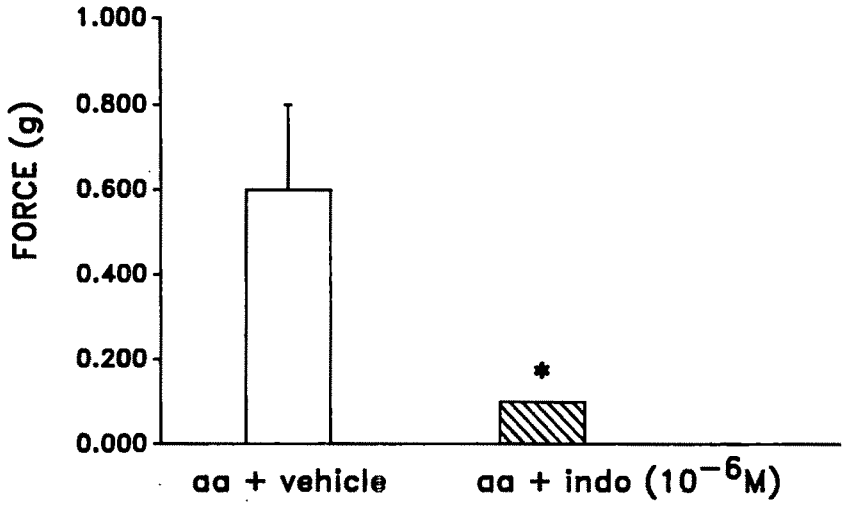


Fig. 2. Response of human chorionic plate arteries to 10^{-5} mol/L arachidonic acid *(aa)* in the presence of 10^{-6} mol/L indomethacin *(indo)* ($n = 4$) or its vehicle ($n = 7$). Data expressed as mean active force (grams) ± SEM. *Asterisk*, Significant difference ($p < 0.05$) between vehicle and indomethacin.

Student t test with a Bonferroni correction for multiple comparisons. In all comparisons, a probability value of $p < 0.05$ was considered to be statistically significant.

Results

The responses of isolated rings of human chorionic plate arteries to angiotensin II in the presence of indomethacin or its vehicle are summarized in Fig. 1. After the first exposure to angiotensin II, successive applications (second through fourth doses) produced significantly less contractile force than the original application (tachyphylaxis). Tachyphylaxis to angiotensin II was similar in the vehicle and indomethacin-treated groups.

The responses of human chorionic plate arteries to exogenous arachidonic acid in the presence of 10^{-6} mol/L indomethacin or its vehicle are summarized in Fig. 2. In these experiments, 10^{-4} mol/L arachidonic acid produced a significant contraction of the vessel, which was prevented by inhibition of cyclooxygenase with 10^{-6} mol/L indomethacin.

Fig. 3 compares the active force in K^+-contracted arteries exposed to iloprost (10^{-5} mol/L) or sodium nitroprusside (10^{-5} mol/L). In these studies arteries contracted with 40 mmol/L K^+ in the absence of iloprost or sodium nitroprusside (K^+ time controls) exhibited a pronounced reduction in active force with time, which was presumably due to stress relaxation of the vessel. Active force in vessels exposed to iloprost was not significantly different from that in K^+ time controls, whereas sodium nitroprusside caused a complete relaxation of the arteries.

Comment

Vasodilator prostaglandins, particularly PGI_2, appear to play an important role in the control of vascular reactivity, platelet aggregation, and renal and uteroplacental blood flow during normal pregnancy.[12] A major role of prostaglandins in pregnancy may be to maintain a low peripheral vascular resistance and to protect against the potential hypertensive effects of the elevated plasma angiotensin levels that occur in normal pregnancy.[13] If the interaction between vasodilator prostaglandins and angiotensin II is somehow disturbed, pregnancy-induced hypertension may develop, posing the threat of morbidity and mortality for the patient and her fetus.[14]

A number of studies have suggested that angiotensin II stimulates the synthesis and release of PGI_2 and other prostanoids[6, 7, 15-19] and that endogenous prostaglandins may modulate the response of vascular smooth muscle to angiotensin II.[5-7] However, other studies[8, 9] suggest that vasodilator eicosanoids do not contribute to angiotensin tachyphylaxis. In the current experiments, we compared successive angiotensin II–induced contractions of human chorionic plate arteries in the presence and absence of indomethacin to determine whether the release of cyclooxygenase products from the vessel modulates angiotensin II–induced contractions of these arteries.

In these experiments the contractile response of the arteries to successive applications of the same concentration of angiotensin II was significantly less than the initial response (angiotensin II tachyphylaxis). Vessel responses were virtually identical in the presence and absence of indomethacin. The latter observation is con-

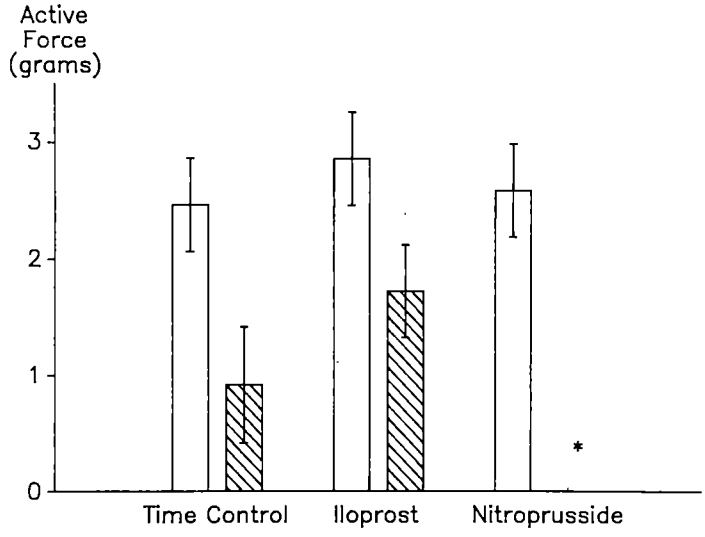


Fig. 3. Response of K^+-contracted human chorionic plate arteries to stable prostacyclin analog iloprost (10^{-6} mol/L) and sodium nitroprusside (10^{-5} mol/L). *Open bars*, Active force developed during initial activation of vessel with 40 mmol/L K^+; *cross-hatched bars*, active force during exposure to iloprost ($n = 11$), nitroprusside ($n = 5$), or 40 mmol/L K^+ with no vasodilator agent *(Time control)* ($n = 7$). Data are expressed as mean active force (grams) ± SEM. *Asterisk*, significant difference ($p < 0.05$) from active force measured in presence of iloprost. See text for details.

sistent with the view that liberation of vasodilator products of the cyclooxygenase pathway does not modulate angiotensin II–induced contractions of these fetal vessels.

Our observation that iloprost did not relax K^+-contracted chorionic plate arteries suggests that PGI_2 would not contribute to angiotensin II tachyphylaxis of these vessels, even if it is liberated from the arteries in response to angiotensin II. The lack of a dilator response to iloprost that we observed in the current experiments is consistent with the results of several animal studies, which have demonstrated that prostacyclin does not relax sheep[20-22] or rabbit[23] placental vessels and does not reverse angiotensin II–induced constriction in the sheep placental vasculature.[24, 25]

Finally, we observed that exogenous arachidonic acid caused a contraction of the arteries. This contraction was blocked by indomethacin. Although vasoconstrictor responses to arachidonic acid have been reported in some blood vessels (predominantly veins),[26, 27] arachidonic acid produces a relaxation of most arteries,[26-28] apparently as a result of the production of PGI_2 by the vascular endothelium.[28] Our observation that human chorionic plate arteries exhibit contractile responses to arachidonic acid is consistent with Tulenko's report that this compound causes constriction of resistance arteries supplying the exchange villi of the human fetal placenta.[1] Taken together, these observations indicate that the cyclooxygenase pathway in these vessels produces vasoconstrictor substances rather than vasodilator compounds.

Likely candidates for cyclooxygenase-dependent constrictor substances include thromboxane A_2,[3, 12, 29, 30] prostaglandins that have constrictor actions on chorionic plate arteries, e.g., prostaglandin E_2 or $F_{2\alpha}$,[1-4, 31] prostaglandin endoperoxides,[32] and superoxide anions generated by the hydroperoxidase activity of prostaglandin H synthase.[33, 34] Regardless of the specific compounds produced, our observation that exogenous arachidonic acid produces an indomethacin-sensitive constriction of chorionic plate arteries provides additional support for the hypothesis that angiotensin II tachyphylaxis in these vessels is not due to the production of vasodilator products by the cyclooxygenase pathway.

Although the results of the present study are consistent with the hypothesis that vasodilator products of the cyclooxyenase pathway are not direct modulators of angiotensin II–induced contractions of human chorionic plate arteries, our observations do not preclude the possibility that a reduced production of PGI_2 from other sources, e.g., uterus[35] or fetal vessels,[5] may indirectly enhance the reactivity of maternal vessels to angiotensin II in pregnancy-induced hypertension by reducing the overall level of vasodilator influences operating on these vessels. Further studies designed to identify the mechanisms of angiotensin tachyphylaxis and vascular control in the maternal and umbilicoplacental circulations should provide valuable insight into

circulatory control during normal pregnancy and pregnancy-induced hypertension.

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Perihepatic adhesions: Not necessarily pathognomonic of pelvic infection

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Perihepatic adhesions are often considered to be associated with pelvic inflammatory disease and subsequent infertility. Seventeen patients out of 100 undergoing elective laparoscopic sterilization had evidence of perihepatic adhesions. Four of the 17 showed evidence of old pelvic inflammatory disease, but only two gave a history of sexually transmitted disease. All patients had a negative gonorrhea culture and 50 also had a negative chlamydia culture before the procedure. These data suggest that perihepatic adhesions may be observed in otherwise normal fertile women, and its presence does not necessarily imply pelvic inflammatory disease or poor fertility. (Am J Obstet Gynecol 1992;167:115-6.)

Key words: Perihepatic adhesions, Fitz-Hugh–Curtis syndrome, pelvic inflammatory disease, laparoscopic tubal ligation

Perihepatic adhesions are a well known sequelae of pelvic inflammatory disease. Curtis[1] in 1930 and Fitz-Hugh[2] in 1934 put forth the popular concept of a perihepatitis syndrome associated with gonococcal pelvic inflammatory disease; the syndrome is not well known under the eponym combining their names. *Neisseria gonorrhoeae* and chlamydia are considered to be the principal agents involved in the pathogenesis of Fitz-Hugh–Curtis syndrome.[1-3]

In this article we present a series of patients who at the time of laparoscopic tubal ligation were also assessed for the presence of perihepatic adhesions. A study of operative findings presents a unique new subgroup of patients with Fitz-Hugh–Curtis syndrome.

Material and methods

One hundred consecutive patients undergoing elective laparoscopic tubal ligation were enrolled in this study. All patients had a negative *N. gonorrhoeae* culture, and the last 50 also had a negative chlamydia culture within 6 weeks of the surgery. A standard history included questions concerning history of sexually transmitted diseases, pelvic inflammatory disease, liver disease, or gastrointestinal diseases. All patients underwent routine laparoscopic tubal ligation. After evaluation of the pelvis for evidence of pelvic inflammatory disease and tubal ligation, evaluation of the liver capsule and anterior abdominal wall was carried out.

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Table I. Perihepatic adhesions: historic factors and operative findings

	Adhesions (n = 17)	*No adhesions (n = 83)*
History of sexually transmitted disease	2 (11.8%)	12 (14.5%)
History of pelvic infection	0	2 (2.4%)
Evidence of chronic pelvic infection	4 (23.5%)	5 (6.0%)

The patients were divided into two groups on the basis of the operative findings: those with and those without perihepatic adhesions.

Results

All patients were clinically asymptomatic and had normal results of abdominal and pelvic examinations before surgery. There was no statistically significant difference in age or parity in the two groups of patients.

Seventeen patients had evidence of perihepatic adhesions. Two of the patients in the adhesion group (11.8%) and 12 of the patients in the no-adhesion group (14.5%) gave a history of sexually transmitted diseases. No patient with adhesions had a documented episode of pelvic inflammatory disease (Table I). Four of the 17 patients (23.5%) with adhesions also had evidence of old pelvic infection. This was much higher than the patients who had no perihepatic adhesions but had similar pelvic findings (five of 83 or 6%). Two patients with a history of sexually transmitted disease and perihepatic adhesions also had evidence of old pelvic infection.

Comment

Our study documents the presence of perihepatic adhesions in a new group of patients, namely, fertile asymptomatic patients with no laparoscopic evidence of old pelvic infection. None of the patients had a history of pelvic infection, and only two had a history of sexually transmitted disease. Thirteen patients had no evidence of prior infection in the pelvis, and the presence of perihepatic adhesions was a surprising finding in this group. Computer-assisted search of the literature did not reveal any documentation of such a series.

The current study suggests that perihepatic adhesions may be the only residual effect left from a pelvic infection or may occur without prior pelvic infection. It also suggests that the mere presence of perihepatic adhesions is not an indication of unfavorable outcome in patients with infertility, because all our patients were fertile women undergoing interval sterilization. A larger study to define the possible causes of perihepatic adhesions is under way.

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Prophylactic amnioinfusion improves outcome of pregnancy complicated by thick meconium and oligohydramnios

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OBJECTIVE: The null hypothesis is that the use of intrapartum amnioinfusion in labors complicated by the presence of thick meconium and oligohydramios will not decrease the incidence of fetal distress, cesarean delivery, meconium aspiration, or meconium aspiration syndrome.

STUDY DESIGN: One hundred seventy term and postterm patients with thick meconium and oligohydramnios were randomly chosen to receive amnioinfusion or standard obstetric care without amnioinfusion. The frequency of fetal distress, cesarean section, meconium aspiration, and meconium aspiration syndrome were subject to χ^2 analysis, Student's t test, or Fisher's exact test.

RESULTS: The rate of fetal distress was significantly reduced in the amnioinfusion group compared with controls (three of 85 vs 19 of 85, relative risk 0.15, 95% confidence interval 0.06 to 0.42). The rate of cesarean section for fetal distress was significantly reduced in the amnioinfusion group (two of 85 vs 17 of 85, relative risk 0.118, confidence interval 0.03 to 0.49). The rates of meconium aspiration (four of 85 vs 33 of 85, relative risk 0.12, confidence interval 0.0449 to 0.327) and meconium aspiration syndrome (0 of 85 vs five of 85, relative risk 0.09, confidence interval 0.009 to 0.872) were significantly reduced by amnioinfusion.

CONCLUSIONS: Amnioinfusion improves the outcome in pregnancies complicated by thick meconium and oligohydramnios. (AM J OBSTET GYNECOL 1992;167:117-21.)

Key words: Amnioinfusion, meconium, oligohydramnios, fetal distress, prolonged pregnancy

The presence of meconium in the amniotic fluid of the fetus in a vertex presentation has been considered to be a sign of fetal stress or distress. Meconium has been associated with increased perinatal morbidity and death, especially when meconium aspiration syndrome occurs.[1-3] Meconium aspiration syndrome develops when mechanical obstruction and chemical inflammation occur as a result of aspiration of meconium into the lower respiratory tract of the fetus or the neonate. The syndrome is defined as respiratory distress in a neonate with meconium aspiration. There is a mortality rate of approximately 25%, and it accounts for 2% of all perinatal deaths.[4]

Meis et al.[3] evaluated patients with meconium passage during early and late labor and graded the presence of meconium as light or heavy. Heavy meconium passage early in labor was associated with increased fetal morbidity, including abnormal fetal heart rate (FHR) patterns (late decelerations, severe variable decelerations, and decreased variability), prolonged second stage of labor, and an increased incidence of operative intervention (including operative forceps deliveries and cesarean section). A significantly higher incidence of neonatal morbidity and mortality, particularly in association with meconium aspiration, occurred when heavy meconium was present early in labor.

In 1976 Carson et al.[5] described the combined obstetric-pediatric approach to infant tracheal suctioning at delivery. In spite of a decrease in the incidence of meconium aspiration syndrome, the neonatal mortality incidence has remained at 2%.[4]

Wenstrom et al.[6] randomly compared 85 patients with thick meconium. The study group was infused with 1000 ml of normal saline solution initially and every 6 hours until delivery. The control patients received routine management. The incidences of low 1-minute Apgar score, meconium below the cords, and operative delivery were significantly less in patients receiving amnioinfusion. The three cases of meconium aspiration syndrome occurred in patients receiving routine management. Sadovsky et al.[7] demonstrated that 19 cases of labor treated with amnioinfusion for thick meconium had a better neonatal outcome com-

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Table I. Patient demographic characteristics

	Infusion group (N = 85)	*Control group (N = 85)*	*Significance*
Age (yr, mean ± SD)	22.6 ± 3.6	22.3 ± 3.4	NS
Parity (mean ± SD)	0.5 ± 0.6	0.4 ± 0.7	NS
No. of nulliparous women	59 (69%)	52 (61%)	NS
No. of multiparous women	26 (31%)	33 (39%)	NS
Estimated gestational age (days, mean ± SD)	294.0 ± 6.0	293 ± 8.0	NS
No. of patients with postdates pregnancies (>287 days)	70 (82%)	76 (89%)	NS
Amniotic fluid index (cm, mean ± SD)	3.5 ± 0.7	3.4 ± 0.8	NS
Dilatation of cervix (cm, mean ± SD)	3.2 ± 0.9	3.3 ± 0.9	NS
No. of patients with spontaneous rupture of membranes	34 (40%)	41 (48%)	NS

NS, Not significant.

pared with 21 cases treated routinely. The frequency of neonatal acidemia and positive pressure ventilation were significantly decreased in the infused group. No newborn infants in the amnioinfusion group had meconium greater than "trace" below the vocal cords.

The purpose of our prospective, randomized clinical trial was to evaluate the possible effects of amnioinfusion with respect to (1) the incidence of intrapartum fetal distress, (2) the incidence of cesarean delivery for fetal distress, (3) Apgar scores, (4) umbilical artery pH values, (5) the incidence of meconium below the vocal cords, and (6) the incidence of meconium aspiration syndrome.

Material and methods

During the 14 months from Sept. 1, 1989, through Oct. 31, 1990, women in labor with thick meconium and oligohydramnios (amniotic fluid index ≤5 cm) were evaluated for participation in the study. The study was approved by the Institutional Review Board of Los Angeles County/University of Southern California. A computer program, Epi-stat, ver 3.0 (copyright 1984) (Tracy L. Gustafson, MD, Round Rock, Tex.), was used to generate the random allocation sequence used to assign subjects to one of two groups: amnioinfusion or standard obstetric care without amnioinfusion. The assignments were placed in sealed, opaque envelopes and opened sequentially after a subject was enrolled.

Criteria for entry included singleton gestation, vertex presentation, gestational age of ≥37 weeks, estimated fetal weight of ≥2500 gm, cervical dilation of ≤5 cm, normal baseline FHR, the presence of at least one acceleration of ≥15 seconds duration and ≥15 beats above the baseline, ruptured membranes (artificial or spontaneous), thick meconium, and amniotic fluid index ≤5 cm.

Patients were excluded for any of the following reasons: abnormal FHR patterns including baseline tachycardia >160 beats/min or bradycardia <120 beats/min, persistent late decelerations, and moderate or severe variable decelerations. Other reasons for exclusion included vaginal bleeding, nonvertex presentation, chorioamnionitis, fetal anomalies, uterine anomalies, or contraindication to labor.

Patients meeting these criteria and giving informed written consent were then randomized to receive either amnioinfusion or standard obstetric care.

All participants had routine admission history, physical examination, and laboratory tests performed. Meconium, identified as thick, noted at any time, including that present on admission or noted later after spontaneous or artificial rupture of membranes, was the primary criterion for entry to the study. Meconium was clinically defined as being thick if it appeared thick, tenacious, and opaque; had a "pea soup" quality; and contained significant particulate matter, after Wenstrom and Parsons.[6] One of the authors confirmed the presence of thick meconium and performed an ultrasonographic examination with a 3.5 or 5 MHz linear-array transducer (Ultramark 4, Advanced Technology Laboratories, Bothell, Wash.). The amniotic fluid index was determined, and a singleton, vertex, anatomically normal fetus was confirmed ultrasonographically.

An intrauterine pressure catheter and fetal scalp electrode provided continuous monitoring of intrauterine pressure and the FHR. Amnioinfusion was performed in a manner that was a modification of that outlined by Miyazaki and Nevarez[8] and Strong et al.[9] A total of 500 ml of normal saline solution, warmed to 37° C, was infused by gravity flow through an intrauterine pressure catheter over 20 to 30 minutes. External uterine tocodynamometry was used during the actual infusion process.

After completion of the amnioinfusion, the amniotic fluid index was determined. If the postinfusion index was <5 cm, an additional 500 ml was infused after ensuring correct catheter placement. If the amniotic fluid index was >10 cm, the patient was observed. If the subsequent index was >5 cm and <10 cm, an additional 250 ml of normal saline solution was infused. Repeat amniotic fluid index determinations were performed hourly by the investigators until delivery.

Clinical management and FHR pattern interpretation, including the diagnosis of fetal distress, was per-

formed by the resident physicians and consultant staff. Customary obstetric management was performed in both groups of patients. DeLee suctioning of the oropharynx and nasopharynx was performed on all infants after delivery of the infant's head. Umbilical cord artery and vein blood gases were obtained in all cases. A pediatric resident attended all deliveries because of the known presence of thick meconium. The neonatal attendants were unaware if the neonate was in the amnioinfusion or control group. The presence or absence of meconium in the oropharynx and below the vocal cords was evaluated in all neonates.

Outcome parameters included (1) fetal distress, (2) operative intervention rates (cesarean delivery, forceps, or vacuum delivery), (3) operative intervention for fetal distress, (4) presence of meconium in the oropharynx or below the vocal cords, (5) meconium aspiration syndrome, (6) umbilical artery pH, (7) and Apgar scores. Fetal distress was defined as bradycardia, repetitive severe variables, or repetitive late decelerations as described by Kubli et al.[10]

A p value of 0.05 was used to establish statistical significance. Assuming a β error of 0.2, a power calculation suggested that a minimum of 79 patients should be assigned to each study group. The power calculation was based on the differential emergency cesarean delivery rate of the Wenstrom and Parsons[6] study (3% vs 16%, respectively).

Statistical comparisons were performed with the Student t test, χ^2, or Fisher's exact test and the calculation of relative risks with 95% confidence intervals.[11]

Results

Informed consent was obtained for 170 patients who met the entry criteria. Eighty-five subjects were randomized into each group. After enrollment no subject was excluded from the study or from the analysis. The treatment and control groups were similar for maternal age, parity, gestational age, and amniotic fluid index (Table I). The groups did not differ with respect to the proportions of nulliparous women, postdates pregnancies (estimated gestational age >287 days), or patients with intact membranes at the time of admission (Table II). All patients exhibiting thick meconium had an amniotic fluid index <5 cm.

The treated group required an average of 1.14 infusion sequences to attain an initial amniotic fluid index value >10 cm. Seventy-two of 85 (85%) patients required a single amnioinfusion, 12 patients received two, and one received three. The total volume required ranged from 500 to 1250 ml. The mean (±SD) was 544 ± 110 ml (Table III). Nulliparous and multiparous patients did not differ statistically with respect to the initial infusion volumes required.

Reinfusions were categorized as follows: (1) part of the initial infusion process when the initial infusion of 500 ml did not restore the amniotic fluid index to 10 cm or (2) later reinfusions when the amniotic fluid index decreased below 10 cm during the course of labor. A total of 13 of 85 (15%) required more than one amnioinfusion; six of 13 immediately after the initial amnioinfusion and seven of 13 at some point later in labor. The initial 500 ml infusion increased the amniotic fluid index to ≥10 cm in 79 of 85 (93%) patients. The initial infusion did not increase the amniotic fluid index to 10 cm in six patients, and fluid was visibly leaking from the vagina in these cases. In this situation, the correct placement of the uterine catheter was confirmed and reinfusion successfully increased the amniotic fluid index to ≥10 cm. Subsequent reinfusion was required later in labor in seven patients: two at 3 hours, three at 4 hours, one at 6 hours, and one at 7 hours (Table III). No reinfusions were required before 3 hours from the initial infusion once an amniotic fluid index of 10 had been achieved.

Table II. Distribution of high-risk patients

	Infusion group (N = 85)	*Control group (N = 85)*	*Significance*
Hypertension–pregnancy-induced hypertension	10 (12%)	13 (15%)	NS
Diabetes	5 (6%)	3 (4%)	NS
Previous cesarean delivery	11 (13%)	9 (11%)	NS
Postdates pregnancies (>287 days)	70 (82%)	76 (89%)	NS
Drug abuse	1 (1%)	0	NS
History of pyelonephritis	3 (4%)	2 (2%)	NS

NS, Not significant.

The initial 500 ml infusion increased the amniotic fluid index 8.2 ± 2.2 cm. The smaller infusion volume of 250 ml raised it 4.2 ± 1.5 cm.

The overall operative interventions were 30 of 85 (36%) in the amnioinfused group versus 47 of 85 (55%) in the control group ($p < 0.05$). The amnioinfusion group demonstrated less fetal distress in labor, three of 85 versus 19 of 85 (relative risk 0.16, 95% confidence interval 0.06 to 0.42), and a lower incidence of cesarean delivery for fetal distress, two of 85 versus 17 of 85 (relative risk 0.118, 95% confidence interval 0.03 to 0.49). Cesarean birth, for arrest disorders, were similar in the amnioinfusion group and the control group, 11 of 83 (13%) versus eight of 68 (12%) (relative risk 0.88, 95% confidence interval 0.38 to 2.08) (Table IV).

The mean birth weight of the infused and control groups, 3505 ±336 gm and 3573 ±332 gm, were not significantly different. No adverse effects of amnioin-

Table III. Amnioinfusion characteristics

Variable	*All patients*	*Nulliparous patients*	*Multiparous patients*	*Significance**
Volume infused (ml, mean ± SD)	544 ± 110	548 ± 99	538 (127)	NS
Proportion of patients receiving reinfusion	13/85 (15%)	10/52 (20%)	3/34 (9%)	NS

**NS*, Not significant.
Value for *t* test comparing means between nulliparous and multiparous patients.

Table IV. Intrapartum operative interventions

	Infusion group (N = 85)	*Control group (N = 85)*	*Significance*
Operative delivery for fetal distress			
Cesarean	2 (2%)	17 (20%)	$p < 0.001$
Forceps	0	0	NS
Vaccum	1 (1%)	2 (2%)	NS
TOTAL	3 (4%)	19 (22%)	$p < 0.05$
Operative delivery for other indications			
Cesarean	11 (13%)	8 (9%)	NS
Forceps	9 (11%)	11 (13%)	NS
Vacuum	7 (8%)	9 (11%)	NS
TOTAL	27 (32%)	28 (33%)	NS
Total operative interventions			
Cesarean	13 (15%)	25 (29%)	$p < 0.001$
Forceps	9 (11%)	11 (13%)	NS
Vacuum	8 (9%)	11 (13%)	NS
TOTAL	30 (35%)	47 (55%)	$p < 0.05$

NS, Not significant.

fusion were observed. Specifically, no cases of cord prolapse, uterine hypertonus, or acute fetal distress during infusion were noted. The overall rate of infectious morbidity was not significantly different between the amnioinfusion and control groups. Specifically, the incidence of choricamnionitis and intrapartum fever was similar in the amnioinfusion group and control group, seven of 85 (8%) versus four of 85 (5%) (relative risk 0.68, 95% confidence interval 0.53 to 5.76). The occurrence of endoparametritis after cesarean birth was not significantly different between the amnioinfusion group and the control group, four of 13 (31%) versus seven of 25 (28%) relative risk 1.11, 95% confidence interval 0.39 to 3.08). The incidence of endoparametritis after vaginal delivery was not different when comparing the amnioinfusion group and control group, four of 72 (6%) versus two of 60 (3%), respectively (relative risk 1.67, 95% confidence interval 0.36 to 8.79).

The amnioinfusion group had a fewer neonates with meconium in the oropharynx, 29 of 85 versus 70 of 85 (relative risk 0.41, 95% confidence interval 0.31 to 0.54), and significantly fewer with meconium below the vocal cords, four of 85 versus 33 of 85 (relative risk 0.12, 95% confidence interval 0.056 to 0.26). No neonates in the amnioinfusion group had meconium aspiration syndrome compared with five neonates in the control group (relative risk 0.09, 95% confidence interval 0.09 to 0.872). All five underwent operative delivery for fetal distress, four by cesarean delivery and one by vacuum extraction. All five infants with meconium aspiration syndrome had an umbilical artery pH of <7.10 and demonstrated a mixed acidosis.

The mean (±SD) umbilical artery pH value of the infusion group was significantly higher than that of the control group, 7.27 (0.05) versus 7.22 (0.09), $p = 0.002$. The mean (±SD) umbilical arterial base deficit of the infusion group was significantly less than the control group, five (3.8) verus seven (4.8), $p < 0.01$. Furthermore, the proportion of infants with an umbilical artery pH value <7.20 was significantly less in the amnioinfusion group, seven of 85 (8%), compared with the control group, 31 of 85 (36%), $p < 0.001$.

There were seven infants in the amnioinfusion group with a umbilical artery pH value <7.20. Three of these seven (43%) had a base deficit of ≤10, consistent with a respiratory acidosis. The remaining four (57%) infants had a base deficit of ≥10, consistent with a mixed acidosis. None of the seven infants required care in the neonatal intensive care unit, and all were discharged home after an uncomplicated postnatal course.

There were 31 infants in the control group with an umbilical artery pH value <7.20. Thirteen (42%) had a base deficit of ≤10, consistent with a respiratory acidosis, and 18 (58%) had a base deficit >10, consistent with a mixed acidosis.

Nineteen patients in the control group developed fetal distress in labor. Seventeen of the 19 were delivered by cesarean section. The other two were delivered by vacuum extraction. Twelve of 17 (71%) who underwent cesarean delivery had an umbilical artery pH <7.20.

The proportion of infants with a 1-minute Apgar score <7 was significantly less in the amnioinfusion group, six of 85 (7%) compared with 26 of 85 (31%) in the control group, $p < 0.001$; the proportion of infants with a 5-minute Apgar score <7 was also significantly less, 0 of 85 compared with eight of 85 (9%) in the control group, $p < 0.001$.

Comment

In this study the presence of thick meconium in labor was 100% specific in identifying patients with oligohydramnios. The observation of thick meconium may thus be associated with decreased amniotic fluid volume, and amnioinfusion should be considered. The amnioinfusion technique used in our study was simple to accomplish, did not require special equipment, had no adverse effects, and was always successful. The effect of amnioinfusion in this study was consistent with that described by Strong et al.,[12] who demonstrated a ±SD mean increase of 4.3 ± 1.5 cm in the amniotic fluid index after the infusion of 250 ml of normal saline solution. The infusion of 500 ml of normal saline solution increased the amniotic fluid index by a mean ±SD of 8.2 ± 2.2 cm.

This study confirms that of Wenstrom and Parsons[6] in that amnioinfusion decreased the incidence of meconium below the vocal cords of infants delivered after labor complicated by thick meconium. Their study did not show a statistically significant decrease in meconium aspiration syndrome, which was likely due to a small sample size. In our study, the occurrence of meconium aspiration syndrome was significantly increased in the control group. This relatively high incidence is probably attributable to the selection process requiring the presence of both thick meconium and oligohydramnios.

Our study with amnioinfusion improved the outcome of pregnancy complicated by thick meconium and oligohydramnios by reducing operative delivery for fetal distress. Furthermore, neonatal outcome was enhanced by the use of this simple intrapartum management technique. Specifically, prophylactic use of amnioinfusion was associated with decreased fetal distress, a decreased presence of meconium below the vocal cords, and an absence of meconium aspiration syndrome.

Although we have demonstrated benefits of this treatment in a highly selected patient group, unanswered questions remain. What is the role of amnioinfusion in the fetus who has FHR pattern abnormalities and thick meconium? In our study design we purposely excluded fetuses with FHR abnormalities to better assess the single factor of thick meconium. The potential benefit of amnioinfusion in relieving severe variable decelerations is well known. Future studies should evaluate the potential role of amnioinfusion wherein multiple indications such as FHR abnormalities, thick meconium presence, and oligohydramnios are encountered.

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Spontaneous ovarian hyperstimulation syndrome concomitant with spontaneous pregnancy in a woman with polycystic ovary disease

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Ovarian hyperstimulation syndrome has been described after treatment with exogenous gonadotropins, clomiphene citrate, and gonadotropin-releasing hormone. Spontaneous ovarian hyperstimulation syndrome has not been described before, except in association with hypothyroidism. We report on a case associated with spontaneous pregnancy, occurring in a woman with polycystic ovary disease. (Am J Obstet Gynecol 1992;167:122-4.)

Key words: Spontaneous hyperstimulation, pregnancy, polycystic ovary disease

A case of ovarian hyperstimulation syndrome in association with pregnancy and polycystic ovary disease is reported.

Case report

A 19-year-old woman, gravida 1, para 0, was admitted to the hospital because of abdominal pain at 10 weeks' gestation. Her medical and gynecologic history was unremarkable. Menarche occurred at the age of 14 years, and menses have subsequently been regular. Her last menstrual period occurred 10 weeks before admission. She was receiving no medication, including oral contraceptives. Physical examination revealed a lean-appearing young woman with normal blood pressure, pulse, and temperature. The abdomen was mildly distended with tenderness and palpable masses in both lower quadrants. Vaginal examination revealed a normal cervix, a uterus enlarged to 10 weeks' gestation, and ovaries on both sides enlarged 10 cm each and tender. A pelvic sonogram revealed an intrauterine sac with fetal pole and heartbeat. The Crown-rump length was 40 mm, corresponding to a 10 weeks' gestation. The ovaries were multilobulated, measured 9 and 10 cm in diameter, and contained cystic structures (Fig. 1). A significant amount of free fluid was observed in the pouch of Douglas. Laboratory results included human chorionic gonadotropin levels 52,000 mIU/ml, hemoglobin 11.0 gm/dl, hematocrit 38.0%, follicle-stimulating hormone 3.4 mIU/ml, luteinizing hormone 300 mIU/ml, prolactin 32 ng/ml, estradiol 5000 pg/ml, progesterone 48 ng/ml, testosterone 1.8 ng/ml, and cholesterol 194 mg/dl. Liver and kidney function test results were within normal limits. Elective termination of pregnancy was undertaken the day after admission. Three months later, the patient was reexamined. Results of physical examination were unremarkable. Repeat ultrasonography revealed a uterus of normal size. Both ovaries were of normal size, 3 and 4 cm in diameter, with a typical appearance of polycystic ovary disease (i.e., polymicrofollicular reactions of the ovaries). There was no fluid in the cul-de-sac (Fig. 2). Laboratory results in early follicular phase included human chorionic gonadotropin negative. Follicle-stimulating hormone 3.2 mIU/ml, luteinizing hormone 14.8 mIU/ml, prolactin 6.7 ng/ml, estradiol 90 pg/ml, testosterone 0.3 ng/ml, dehydroepiandrosterone sulfate 2.6 ng/ml, thyroxine 7.9 μg/dl, triiodothyronine resin uptake 28%, thyroid-stimulating hormone 0.9 μU/ml (within normal limits).

Comment

We report a case of grade 2 ovarian hyperstimulation syndrome (by a combination of multilobulated ovarian cysts, abdominal pain, and free fluid in the cul-de-sac), occurring spontaneously in association with a 10-week gestation. The patient was not treated with any drug, including ovulation induction. Diagnosis was further confirmed by complete resolution of the ovarian hyperstimulation, as demonstrated by clinical, ultrasonographic, and laboratory data. To the best of our knowledge spontaneous ovarian hyperstimulation syndrome has not been described before. Rotmensch and Scommegna[1] have described a case associated with hypothyroidism and suggested a causal link between both conditions. We ruled out any thyroid disturbances. However, our patient showed both laboratory and ultrasonographic characteristics of polycystic ovary disease. The etiologic and pathophysiologic characteristics of ovarian hyperstimulation syndrome are poorly un-

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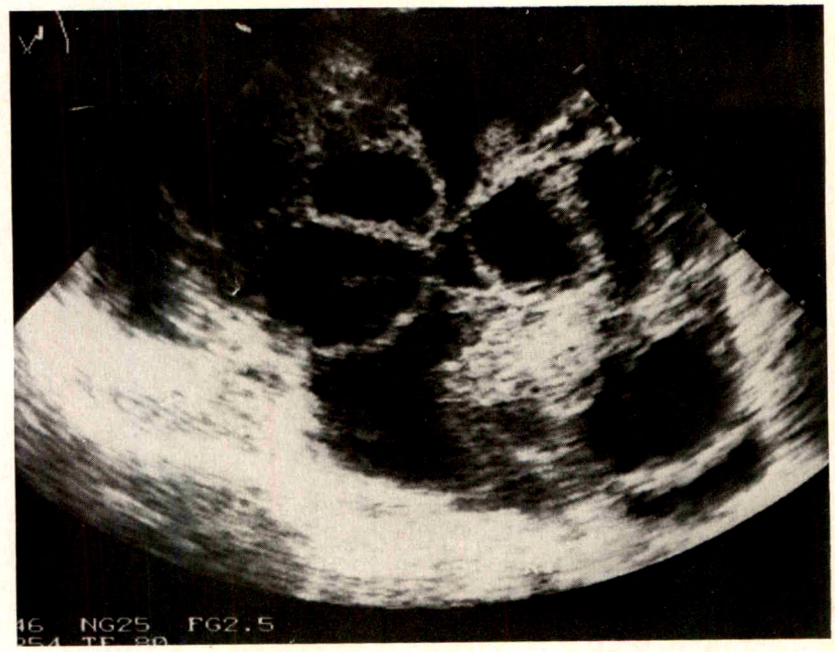


Fig. 1. Transvaginal longitudinal ultrasonographic section showing both ovaries enlarged with multiple cystic structures.

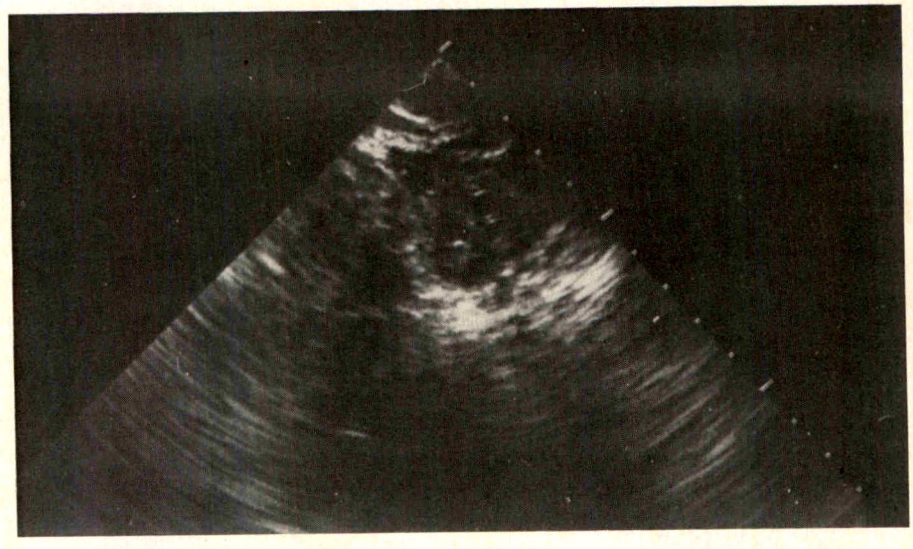


Fig. 2. Transvaginal longitudinal ultrasonographic section showing left ovary with polymicrofollicular reaction.

derstood. Various factors, including estrogen, histamine, prostaglandins, prolactin, aldosterone, and (pro)renin,[2] have been suggested to be involved in the development of the condition. Others found that treatment with a preparation with a high follicle-stimulating hormone/luteinizing hormone ratio causes less ovarian hyperstimulation, and thus ovarian hyperstimulation syndrome might be eliminated by reducing the dosage of luteinizing. Ovarian hyperstimulation syndrome is the result of massive luteinization of a large number of follicles and occurs in 1% to 5% of ovulation-induction treatment cycles. The incidence of hyperstimulation syndrome is higher in women with polycystic ovary disease, probably because of a larger number of follicles ready to respond to stimulation and a higher sensitivity of functional ovarian elements to gonadotropic stimulation. However, clinical experience showed that one of the essential conditions for the development of ovarian hyperstimulation syndrome is the administration of a relatively high dose of human chorionic gonadotropin required for ovulation induction. It has been presumed that the presence of a functioning feedback mechanism

should be adequate to prevent a spontaneous luteinizing hormone surge of height or duration sufficient for luteinizing an excessive number of follicles. Thus withholding of the ovulation-inducing human chorionic gonadotropin injection was advocated as one of the best means for preventing ovarian hyperstimulation.

The case presented suggests that, at least in patients with polycystic ovary disease, endogenous follicle-stimulating hormone is sufficient to stimulate the development of an abnormally large follicular cohort, which may then be luteinized by a spontaneous luteinizing hormone surge. Endogenous human chorionic gonadotropin, secreted by the trophoblast 7 to 8 days after fertilization, could represent an additional factor in sustaining or exacerbating the ovarian hyperstimulation in this case.

Although less common now, ovarian hyperstimulation syndrome still has a very serious impact on the patient's health and may cause severe morbidity. The purpose of our report is to emphasize that, although it is usually a complication of ovulation induction, it can also occur spontaneously.

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Screening for Down syndrome with the femur length/biparietal diameter ratio: A new twist of the data

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OBJECTIVE: The purpose of this study was to determine the value of discordant morphometric measurements as identifiers of Down syndrome by evaluating the relationship of biparietal diameter, femur length, biparietal diameter/femur length ratio, and cephalic index between a group of fetuses with trisomy 21 and a control population.

STUDY DESIGN: Biometric measurements from 48 fetuses with trisomy were reviewed and compared with 107 normal fetuses of similar gestational age. Data were analyzed in 2-week gestational age intervals to determine the effect of gestational age on ultrasonographic detection of Down syndrome. Outcome measures were subject to least-squares linear regression and the *t* test for analysis.

RESULTS: A positive relationship between abnormal morphometric measurements and fetuses with Down syndrome was detected but only during specific weeks of pregnancy.

CONCLUSION: Although it appears that biometric measurements may be useful for Down syndrome, further study is needed before its widespread introduction into clinical practice. (AM J OBSTET GYNECOL 1992;167:124-8.)

Key words: Fetus, Down syndrome, ultrasonography

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Antenatal identification of congenital abnormalities in the fetus has increased over the past decade. As a new generation of ultrasonographic equipment became available, numerous publications[1-7] demonstrated its increasing ability to identify dysmorphic features of the fetus, leading to the antenatal diagnosis of specific genetic syndromes. Recent studies have also suggested

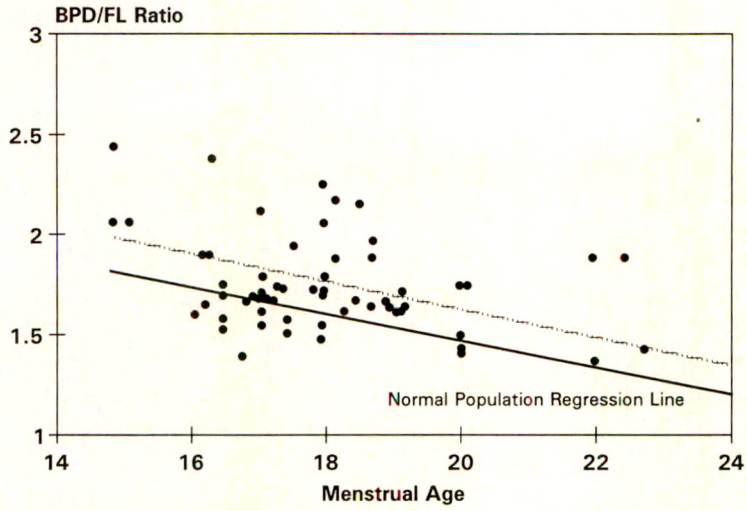


Fig. 1. Scatterplot of individual BPD/femur length *(FL)* ratio measurements versus menstrual age for trisomy 21 population. *Broken regression line*, Patients with trisomy 21; *solid line*, regression line for control population.

that discordant morphometric measurements may be useful in screening for the identification of the fetus at risk of trisomy 21. Reported characteristic features of Down syndrome include (1) brachycephaly,[8] (2) thickened nuchal skin folds,[9] (3) hypoplasia of the middle phalanx with clinodactyly,[10] (4) shortened femur length,[8, 11] (5) abnormal biparietal diameter (BPD) to femur length ratio,[12] and (6) transverse palmar crease (simian).[13]

The importance of identification of these features is potentially significant. Although standard practice dictates the offering of prenatal chromosomal diagnostic procedures to patients at risk for Down syndrome, over 80% of affected fetuses are born to women without historic risk factors. Maternal serum α-fetoprotein screening programs may help to identify 20% of these low-risk women, and potentially a greater percentage of patients will be identified with the addition of serum human chorionic gonadotropin[14-17] and estriol.[18, 19] The majority of these affected fetuses, however, go undetected until birth unless procedures for cytogenetic assessment are offered and used. In an attempt to better identify the fetus at risk for trisomy 21, a retrospective study was undertaken to evaluate the relationship of the BPD, femur length, BPD/femur length ratio, and cephalic index between a large group of fetuses with trisomy 21 and a control population.

Material and methods

Patients seen either at the Prenatal Diagnostic Program, Women's Hospital, Los Angeles County University of Southern California Medical Center, Children's Hospital of Los Angeles, or the Genetics Institute, Pasadena, California, were all eligible for entrance into this study. Medical records from patients with trisomy 21 diagnosed by amniocentesis between July 1985 and June 1988 were reviewed. The ultrasonographic examinations for each of these cases were also evaluated. Ultrasonographic data was obtained prospectively from 107 patients of similar age with normal chromosomes to develop our control population. Gestational age was determined by well-established dates, i.e., dates calculated from last menstrual periods, confirmed by early ultrasonographic examinations. Data from only a single examination were used for analysis of the affected and control populations. The examinations of the fetuses with trisomy 21 were recorded at the time of amniocentesis and before chromosomal diagnosis. The examinations were all carried out by the authors. Either a General Electric (Milwaukee) RT3000 or an ATL/ADR (Bothell, Wash.) Ultra Mark IV was used to make these measurements.

Least-squares linear regression of the menstrual age and the ultrasonographic parameters studied was performed to determine the regression lines for slope and

Table I. Results of comparison between regression lines of each of biometric measurements and overall groups

	Slope	*Intercept*	*Down syndrome vs control*
BPD	$p = 0.0001$	$p = 0.0001$	Decreased Down syndrome
Femur length	$p = 0.0001$	$p = 0.0001$	Decreased Down syndrome
Cephalic index	$p = 0.72$	$p = 0.97$	No difference
BPD/femur length ratio	$p = 0.86$	$p = 0.60$	No difference

Table II. Comparison of biometric measurements of Down syndrome group and control group stratified by 2-week intervals

Menstrual weeks	Study group	Mean				SD			
		BPD	Femur length	Cephalic index	BPD/femur length	BPD	Femur length	Cephalic index	BPD/femur length
15-16.9	Control (n = 22)	3.5	2.0	0.78	1.79	0.21	0.23	0.04	0.15
	Down syndrome (n = 14)	3.5	2.0	0.81	1.82	0.37	0.44	0.05	0.31
17-18.9	Control (n = 50)	4.0	2.5	0.78	1.66	0.23	0.25	0.04	0.12
	Down syndrome (n = 24)	4.0	2.2	0.82	1.79	0.34	0.29	0.04	0.19
19-20.9	Control (n = 22)	4.7	3.1	0.77	1.54	0.24	0.26	0.03	0.11
	Down syndrome (n = 8)	4.2	2.6	0.79	1.63	0.40	0.29	0.03	0.11
21-22.9	Control (n = 13)	5.4	3.7	0.76	1.48	0.30	0.24	0.03	0.09
	Down syndrome (n = 2)	4.9	3.0	0.78	1.66	0.57	0.07	0.07	0.23

intercept between the control and the population with Down syndrome. The two populations were further compared for statistical significance between the intercept and slope of the regression lines for the measurements studied. To further assess the possible relationships between different gestational intervals for the detection of Down syndrome, the data were also analyzed stratified by 2-week intervals from 15 to 23 weeks. A comparison of the mean values of the ultrasonographic measurements between the control and study population was performed with the t test. Statistical significance was noted if the p value was ≤ 0.05.

Results

A total of 48 fetuses with trisomy 21 were identified during this period of time. The slope and intercept for the regression lines of each of the populations is presented in Table I, with the results of the comparison between the regression lines describing the populations. Both BPD and femur length were significantly decreased downward, whereas the cephalic index and BPD/femur length ratio were not significantly different when evaluated with regression analysis. The individual data for the BPD/femur length ratio is plotted in Fig. 1 along with the mean regression lines for the normal and Down syndrome population for visual inspection.

To further delineate this data in which there appeared to be a possible menstrual age effect in a more confined time period, the data was analyzed by 2-week intervals. Table II contains number, mean, standard deviation, and statistical significance for the four measurements of this comparison between the study and control populations. Between 15 and 16.9 weeks menstrual age no significant differences were observed among any of the parameters. Although the BPD was not significantly decreased, the femur length was significantly smaller in the affected population by 17 and 18.9 weeks. The cephalic index and the BPD/femur length ratio were significantly increased. Between 19 and 20.9 weeks both the BPD and femur length were significantly smaller in the population with Down syndrome, but the cephalic index and BPD/femur length showed no significant differences. It should be noted, however, that the sample size of fetuses with Down syndrome in this gestational age range is small, numbering only 10 subjects. Between 21 and 22.9 weeks only the femur length in the Down syndrome group was significantly smaller. As in the preceding group, the sample size in this range is very small.

Comment

In contrast to the previous published reports, this study evaluated ultrasonographic parameters for screening for trisomy 21 at specific gestational ages. In general, the relationship was not equally distributed across these gestational ages. It appears that there is a relationship between ultrasonographic measurements in fetuses with Down syndrome compared with controls only during specific weeks in pregnancy. However, the difference between the mean femur lengths of the study and control fetuses represents only 3 mm at 17 to 18.9 weeks gestation. Variations in femur lengths between the two groups from 15 to 16.9 weeks gestation may be more difficult to demonstrate. This may have contributed to the failure of finding a statistical difference between this ultrasonographic measurement in the earliest group of fetuses as compared with the later ones. The usefulness of the relationship between biometric measurements and gestational age needs to be further evaluated prospectively to determine if there

Significance			
BPD	*Femur length*	*Cephalic index*	*BPD/femur length*
$p = 0.95$	$p = 0.93$	$p = 0.18$	$p = 0.68$
$p = 0.34$	$p = 0.002$	$p = 0.0002$	$p = 0.006$
$p = 0.0001$	$p = 0.0001$	$p = 0.23$	$p = 0.06$
$p = 0.07$	$p = 0.0001$	$p = 0.78$	$p = 0.45$

is a particular gestational age that can be targeted to give patients who are not currently candidates for amniocentesis, or if amniocentesis is indicated and the patient has declined the procedure, an adjusted risk for having a fetus with trisomy 21.

From the data in this study, it is interesting to note that the femur length measurement remains short beyond a period previously reported by Lockwood et al.,[12] whereas the BPD measurement is variable. Decrease in BPD appears to occur later in the gestational ages evaluated. This may reflect selective delayed growth of the brain in fetuses with Down syndrome after 19 weeks. A decrease in growth of the head may initially be partially compensated by brachycephaly, with an increased cephalic index at 17 to 19 weeks. After this time the cephalic index was not significantly different from the control population, and a decrease in the BPD was found. These observations of the variations in head size and configuration in this cross-sectional study need to be evaluated and confirmed in a longitudinal study. This may be important if biometry is considered as a screening tool for risk for trisomy 21.

Although the patient numbers in our study are large when compared with the experience of most institutions, we believe the numbers are not, as yet, sufficient to draw the firm conclusion that an increased BPD/femur length ratio is the best screening test. Because there are a limited number of fetuses ≥19 weeks' gestation, it is difficult to draw firm conclusions about the lack of statistical significance of the BPD/femur length ratio between the Down syndrome fetuses and the controls from 19 to 20.9 weeks and 21 to 22.9 weeks. Although some reports have suggested that the BPD/femur length ratio can be used,[8, 11, 12, 20] other reports[21-25] have disputed these findings. The data presented suggest that there is indeed a benefit of looking at these ratios for the development of risk assessments for the patient scanned in the second trimester.

The intriguing part of the approach to the detection of malformations is that the measurements required are the same used in all ultrasonographic examinations performed by knowledgeable ultrasonographers. The alternative approaches of selective identification of other structural changes seen in Down syndrome, such as the wide-spaced greater toe, hypoplasia of the middle phalanx with clinodactyly, and heart defect, are clearly more difficult to recognize and will be missed in a significant number of patients at risk, even by the more experienced ultrasonographers. Identification of all markers of Down syndrome seems almost impossible unless only the most experienced sonographers perform all examinations, and the amount of time devoted to each examination is dramatically lengthened. As a manpower shortage already exists in ultrasonographic imaging, this approach would seem to preclude this from becoming a reality. Therefore, critical analysis of data readily identified becomes more important. The observation that the BPD/femur length ratio displayed abnormal trends (without significance) at most gestational ages with significance reached at specific ages provides the impetus for further study.

A further consideration of data difference between studies may be a result of either racial or ethnic variation. The data presented in this paper consist of a truly broad-based sample. Among the three centers participating, the patient populations are uniquely different. One group represents a mostly white, middle to upper socioeconomic group, whereas another center represents mostly a middle- to low-income group of patients from Anglo-American, Asian-American, and Mexican-American populations. We were unable to identify any difference in either the control or affected population between these groups.

Accumulating larger data sets also seems warranted. The early work by Lockwood et al.[12] suggested the benefit of using the method when only the BPD/femur length ratio is considered to be abnormal. In that study any measurement >1.5 SDs was considered to be abnormal, whereas most other studies use >2 SDs above the mean as their cut-off. In a later study, these same authors were unable to find a relationship between an abnormal BPD/femur length ratio and trisomy 21 in a different population group. These seemingly conflicting reports further support the need for a large collaborative study on the use of biometric screening for risk assessment for the fetus with trisomy 21. Because of increasing financial constraints being placed on most comprehensive prenatal diagnostic centers, it seems that either a statewide, regional, or national collective

effort program should be instituted to more adequately derive the data necessary to properly counsel patients.

We chose to look at an interval of 3 years of study to reduce confounding variables of different operators and equipment that may affect some of the measurements. Using this approach, we are also limited by the current tendency to reduce gestational age discrepancies between the BPD and femur length. Clearly, if this study were expanded to a large, multicenter study, interinstitutional and intrainstitutional variation in measurement technique would need to be closely monitored.

In summary, it appears that the use of biometric measurements for screening for Down syndrome appears intriguing. The distribution of data represented suggests that large numbers will be required before the widespread introduction into clinical practice. We therefore feel that a prospective, multicenter study of Down syndrome fetuses with matched controls should be instituted under the control of a central data coordinating center.

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PROGRAM

THURSDAY, SEPTEMBER 24, 1992

7:00 A.M.: Registration
7:45 A.M.: Welcome/S. Robert Kovac, M.D.
7:55 A.M.: Stephen H. Cruikshank, M.D. (Scientific Moderator)
8:00 A.M.: Lane J. Mercer, M.D. *"Antibiotic Prophylaxis for Vaginal Surgery—How Much, When"*
8:30 A.M.: S. Joel Joel-Cohen, M.D. *"General Surgical Principles as Applied to Vaginal Surgery"*
9:00 A.M.: George W. Morley, M.D. *"Vaginal Hysterectomy—How I Do It"*
9:30 A.M.: Break
9:50 A.M.: Thomas G. Stovall, M.D. *"Vaginal Hysterectomy—Tips and New Ideas to Include Outpatient Vaginal Hysterectomy"*
10:20 A.M.: John H. Isaacs, M.D. *"The Difficult Vaginal Hysterectomy"*
10:50 A.M.: David S. Chapin *"Combined Coring and Bisecting for Large Uteri"*
11:20 A.M.: Question & Answer Period
Noon: Lunch
1:00 P.M.: Bobby L. Shull, M.D. *"Anterior Vaginal Segment—Function and Anatomy"*
1:30 P.M.: David W. Warrell, M.D. *"Combined Assessment of Pelvic Visceral Function"*
2:00 P.M.: Marvin H. Terry Grody, M.D. *"Normal Vaginal Axis—Importance of Restoration in Pelvic Support"*
2:30 P.M.: Richard C. Bump, M.D. *"Effect of Vaginal Hysterectomy on Lower Urinary Tract Function"*
3:00 P.M.: Break
3:20 P.M.: Stephen H. Cruikshank, M.D. *"Ureteral Anatomy at Vaginal Hysterectomy"*
3:50 P.M.: Roundtable
Moderators: S. Robert Kovac, M.D.—Stephen H. Cruikshank, M.D.
"Vaginal Hysterectomy: To Close Or Not To Close Peritoneum—Transverse or Vertical Vaginal Cuff Closure—How to Support Vaginal Cuff"
George W. Morley, M.D. John H. Isaacs, M.D.
Bobby L. Shull, M.D. Thomas G. Stovall, M.D.
4:30 P.M.: Question & Answer Period
5:00 P.M.: Adjourn
Wine and Cheese Reception/Exhibit Hall

FRIDAY, SEPTEMBER 25, 1992

7:30 A.M.: Update/Stephen H. Cruikshank, M.D.
8:00 A.M.: John H. Isaacs, M.D. *"Complications of Vaginal Hysterectomy and Repair"*
8:30 A.M.: Bobby L. Shull, M.D. *"Technical Aspects and Results of Paravaginal Defects—Including Vaginal Paravaginal Repair"*
9:00 A.M.: David S. Chapin, M.D. *"Teaching Sacrospinous Suspension—Exposing the Ligament So All Can See"*
9:30 A.M.: Break
10:00 A.M.: George W. Morley, M.D. *"Total Colpocleisis as Treatment of Vaginal Prolapse in the Elderly"*
10:30 A.M.: Marvin H. Terry Grody, M.D. *"The Dysfunctional Postoperative Vagina"*
11:00 A.M.: W. Glenn Hurt, M.D. *"Vaginal Vault Prolapse—Treatment Options"*
11:30 A.M.: Question & Answer Period
Noon: Lunch
1:00 P.M.: S. Joel Joel-Cohen, M.D. *"The Story of Vaginal Surgery"*
1:30 P.M.: *"Vaginal Surgeon's Award"*
Presented by: S. Robert Kovac, M.D.
Presented to: S. Joel Joel-Cohen, M.D.
1:35 P.M.: S. Robert Kovac, M.D. *"Surgical Management of Uterine Prolapse in Young Women"*
2:05 P.M.: Lane J. Mercer, M.D. *"Complications of Episiotomy"*
2:35 P.M.: Harry Reich, M.D. *"Laparoscopic Hysterectomy—Definition and Technique"*
3:05 P.M.: Break
3:20 P.M.: Thomas G. Stovall, M.D. *"Laparoscopic Assisted Vaginal Hysterectomy"*
3:50 P.M.: Harry Reich, M.D. *"Laparoscopic Hysterectomy when Vaginal Hysterectomy is Impossible"*
4:20 P.M.: Roundtable
Moderators: S. Robert Kovac, M.D.—Stephen H. Cruikshank, M.D.
"Laparoscopy Assisted Vaginal Hysterectomy—Laparoscopic Assisted Vaginal Hysterectomy—Laparoscopic Hysterectomy: Valuable Methods—Valuable Marketing Tools"
Harry Reich, M.D. Marvin H. Terry Grody, M.D.
Thomas G. Stovall, M.D.
5:00 P.M.: Question & Answer Period
5:30 P.M.: Adjourn
7:00 P.M.: Social Dinner/Dance

SATURDAY, SEPTEMBER 26, 1992

7:30 A.M.: Update/S. Robert Kovac, M.D.
7:45 A.M.: W. Glenn Hurt, M.D. *"Urogynecology—Training and Practice"*
8:15 A.M.: David W. Warrell, M.D. *"Aetiology of Stress Incontinence of Urine and Surgical Management"*
8:45 A.M.: Donald R. Ostergard, M.D. *"Selection of the Appropriate Surgical Procedure"*
9:15 A.M.: Break
9:45 A.M.: Richard C. Bump, M.D. *"Role of Periurethral Bulk Injections in the Treatment of Incontinence"*
10:15 A.M.: Donald R. Ostergard, M.D. *"Why Surgery Cures or Fails"*
10:45 A.M.: David W. Warrell, M.D. *"The Management of Lower Urinary Tract Fistulae"*
11:15 A.M.: Roundtable
Moderators: S. Robert Kovac, M.D.—Stephen H. Cruikshank, M.D.
"Urogynecology: Operative Choices for Recurrent Stress Urinary Incontinence"
David W. Warrell, M.D. Donald R. Ostergard, M.D.
Richard C. Bump, M.D. W. Glenn Hurt, M.D.
Noon: Question & Answer Period
12:30 P.M.: Adjourn

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Rupture of uterine scar with extensive maternal bladder laceration after cocaine abuse

Chaur-Dong Hsu, MD, MPH, Serena Chen, MD, Terry I. Feng, MD, MPH, and Timothy R.B. Johnson, MD

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A relationship between cocaine use and extensive rupture of a uterine scar has not been reported. We report such a case with extensive laceration of the maternal urinary bladder occurring after ingestion of cocaine. (AM J OBSTET GYNECOL 1992;167:129-30.)

Key words: Cocaine abuse, uterine scar rupture, bladder laceration

Cocaine abuse in women of childbearing age has dramatically increased over the past years. Cocaine use and rupture of an unscarred uterus has recently been reported.[1] However, the incidence and severity of uterine scar rupture in cocaine users during pregnancy remains unknown. We report a case of rupture of a uterine scar with extensive laceration of the maternal urinary bladder after cocaine abuse.

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6/1/37253

Case report

A 34-year-old unregistered black woman, para 4-0-1-4, at 36⅗ weeks' gestation was fully dilated and in active labor. Her medical history was positive for heroin and cocaine abuse. She underwent a cesarean section in 1977 at 40 weeks' gestation for an unknown reason. She had three subsequent vaginal deliveries and an elective abortion without complication. Shortly after admission the patient had a spontaneous vaginal delivery of a 3320 gm female infant with Apgar scores of 2 and 5 at 1 and 5 minutes, respectively. The umbilical cord blood gas values were as follows: pH 7.03, P_{O_2} 10 mm Hg, and P_{CO_2} 80 mm Hg. The urine toxicology screen was positive for cocaine metabolites. After delivery the patient complained of chest pain and diffuse abdominal pain. Urine output showed gross blood, and the abdomen was diffusely tender with some rebound to deep palpation. Pelvic examination revealed a

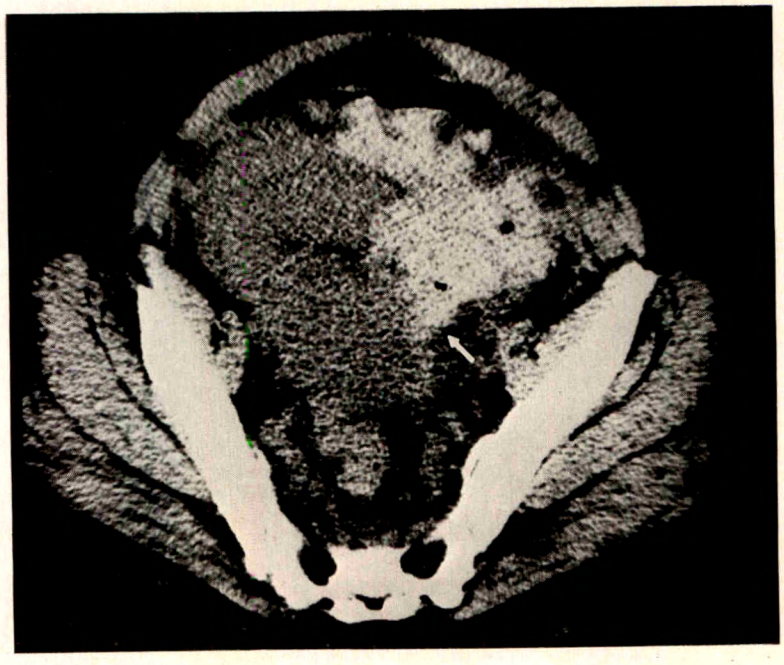


Fig. 1. Uterine rupture: disruption of uterine wall and broad ligament hematoma *(arrow)*.

slightly tender uterus. Blood gas, electrocardiogram, and chest radiographic examination were unremarkable. Ultrasonography was remarkable for a fluid collection to the left of the uterus. Computed tomographic scan (Fig. 1) revealed a large left pelvic hematoma displacing the uterus to the right with small air bubbles within it. A disruption of the wall of the lower uterine segment was suggestive of a uterine rupture. Because of the continued hematuria, a voiding cystourethrogram was obtained and showed immediate intraperitoneal extravasation from the bladder. The patient was then taken for an exploratory laparotomy. A large, left broad ligament hematoma, a large defect in the lower uterine segment, and a large defect in the bladder were noted. A total abdominal hysterectomy and repair of the large posterior bladder defect were performed. The patient did well postoperatively.

Comment

Cocaine use has been implicated in augmenting the contractile response of the pregnant uterus. It has been theorized that cocaine may block norepinephrine reuptake and stimulate the α-adrenergic contractility of uterine smooth muscle. It is possible that this response may increase the risk of uterine scar rupture in cocaine abusers during pregnancy.

Rupture of the low transverse uterine scar is reported in 0% to 2.3% of planned trials of labor. However, the incidence of rupture of a uterine scar with extensive maternal bladder laceration is unknown. Jones et al.[2] have reported eight cases of rupture of a low transverse uterine scar during a trial of labor. Three cases of maternal bladder lacerations were noted. However, seven of eight patients received either oxytocin for induction or augmentation or prostaglandin E_2 for cervical ripening. One patient had a history of three cesarean sections. These factors may explain a higher incidence of maternal bladder lacerations. Our patient did not receive prostaglandin E_2 or oxytocin during labor and delivery. Rupture of the uterine scar and the extensive maternal bladder laceration were closely related to recent cocaine abuse.

In view of increased rates of cocaine abuse during pregnancy and trials of labor and vaginal birth in recent years, the incidence and severity of rupture of uterine scars and further complications such as extensive maternal bladder laceration among recent cocaine abusers with uterine scars may also increase. Whether routine screening of cocaine abuse before a trial of labor, or other precautions, should be taken in women with cocaine abuse remains to be determined.

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Comparison of the efficacy of different local anesthetics and techniques of local anesthesia in therapeutic abortions

Ellen R. Wiebe, MD
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OBJECTIVES: The objectives of this study were to determine whether carbonated or plain lidocaine provides better pain control during abortions and to determine whether deep injections of lidocaine provide better pain control than regular injections of lidocaine.
STUDY DESIGN: Phase 1 was a prospective, randomized, double-blind trial comparing 10 cm^3 of 2% carbonated lidocaine with plain lidocaine. In phase 2 the plain lidocaine group in phase 1 was compared prospectively with the next group of patients, in whom a new technique of deep injection was used. A pain scale was administered by the counselor after dilatation and at the end of the procedure.
RESULTS: The improvement in pain scores with carbonated lidocaine was 8%. The improvement with the deep injection technique was 25%.
CONCLUSION: The improvement in pain scores with deep injection was clinically significant and is recommended. (Am J Obstet Gynecol 1992;167:131-4.)

Key words: Local anesthesia, therapeutic abortion, pain control

Staff working at freestanding abortion clinics are very aware of the need for better pain control during therapeutic abortions. General anesthesia is not an option for most procedures because it increases cost, morbidity, and mortality.[1-4] A review of the literature revealed no studies of the efficacy of pain managment with various types of local anesthesia or techniques used during therapeutic abortions and very few studies about the pain of therapeutic abortions.[5, 6] Before my study, one of our three clinic physicians used carbonated lidocaine and the other two used plain lidocaine. All used a technique of paracervical block involving three to six injections approximately ½ inch deep. In the first phase carbonated lidocaine was compared with plain lidocaine in a prospective, double-blind manner with pain scales administered after dilatation and after suctioning. The hypothesis was that carbonated lidocaine would work better than plain because it is rapidly absorbed and that the difference would be greatest in the dilatation phase. In September 1990 Stubblefield[7, 8] described his technique of deep injections originally described by Glick,[9] and two of our three clinic physicians decided to change to this technique. Stubblefield used 20 cm^3 1% lidocaine instead of 10 cm^3 2% lidocaine, another change that was also made. The pain scales of the plain lidocaine group of phase 1 of the study were compared with those of the two physicians using the new technique in phase 2. The hypothesis was that the deep injection technique would provide better pain control than the regular technique and that the difference would be greatest for dilatation.

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6/1/36691

Material and methods

My study was conducted at a freestanding abortion clinic. Approval for the study was obtained from the ethics committee of the University of British Columbia. All patients at the clinic were invited to participate; only those unable to understand the consent form or unwilling to sign were excluded. The three clinic physicians each had at least 10 years' experience in performing abortions. Patients who requested sedation received 1 mg of lorazepam sublingually 30 minutes before the procedure. In phase 1, 10 cm^3 2% carbonated lidocaine was compared with 10 cm^3 2% plain lidocaine. Both had 2 mg atropine/50 cm^3 added and were administered in three to six sites approximately ½–inch deep. The nurses prepared and labeled the syringes of lidocaine so that neither the physician, the patient, nor the counselor knew which local anesthetic had been used. The counselor administered the pain scale at the end of dilatation and at the end of the procedure by saying, "On a scale of 0 to 10, where 0 is no pain and 10 is the worst pain you can imagine, what was that like for you?" and recording the patient's answer on a coded form. In phase 2 the physicians changed techniques from injecting 10 cm^3 2% lidocaine (with 2 mg atropine/50 ml) in three to six sites (i.e., 12, 3, and 6 or 12, 2, 4, 6, 8, and 10 o'clock positions) approximately

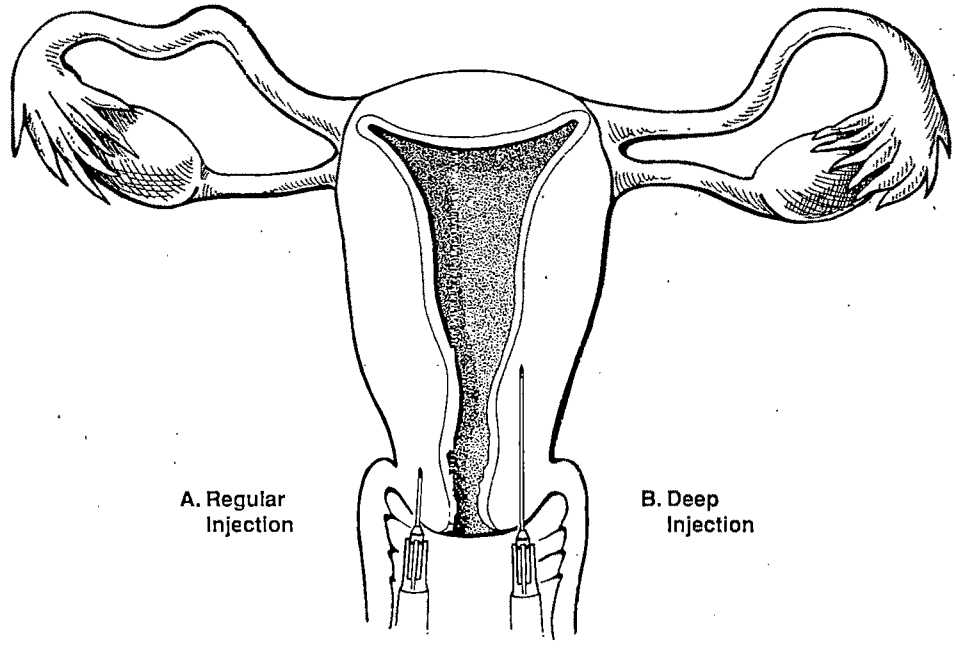


Fig. 1. Position of injections.

Table I. Comparison of pain scales: Phase 1

	Pain scale 1 (mean ± SD)	*Pain scale 2 (mean ± SD)*
Plain lidocaine (n = 81)	5.2 ± 0.3	6.2 ± 0.3
Carbonated lidocaine (n = 86)	4.4 ± 0.3	5.8 ± 0.3
	p = 0.036	p = 0.37

Table II. Comparison of pain scales: Phase 2

Plain lidocaine	*Pain scale 1 (mean ± SD)*	*Pain scale 2 (mean ± SD)*
Deep injection (n = 82)	2.8 ± 0.3	5.2 ± 0.3
Regular injection (n = 81)	5.2 ± 0.3	6.2 ± 0.3
	p = 0.0001	p = 0.018

½–inch deep at the reflection of the vagina off the cervix (Fig. 1, A) to injecting 20 cm³ 1% lidocaine (with 1 mg atropine/50 ml) in the following manner: 1 cm³ was injected in each of six sites (12, 2, 4, 6, 8, and 10 o'clock) superficially to blanch the mucous membrane, then 3 to 4 cm³ was injected 1 to 1.5 inches deep, that is, into the lower uterine muscle in four sites at 4, 6, 8, and 10 o'clock. The syringes were placed in the deep position and aspirated for blood; then a bolus injection was given (Fig. 1, B). Needle extenders were used. One physician used 1-inch, 25-gauge needles, and the other used 1½-inch, 25-gauge needles. There was no delay between the injections and the start of the procedure. Preoperative anxiety was assessed by the counselor in her initial interview and rated as low,[1] moderate,[3] or high.[5] Pain scales were compared with two-sample t tests of means. Two-sample t tests and χ^2 tests were also used to compare study groups on the

Table III. Phase 1

	Plain lidocaine	*Carbonated lidocaine*
Lorazepam		
Yes	45	44
No	14	16
Mean preoperative anxiety score	3.0	3.0
Mean maternal age (yr)	25.8	27.5
Mean gestational age (wk)	8.7	8.7

Table IV. Mean pain scale by different physicians

	Phase 1			*Phase 2*		
Physician No.	*Pain scale*	*Mean*	*No.*	*Pain scale*	*Mean*	*No.*
1	1	5.24	105	1	2.72	54
	2	5.91		2	5.72	
2	1	4.19	51	1	3.21	24
	2	6.47		2	5.12	
3	1	3.41	11			
	2	4.68				

Table V. Effect of lorazepam on pain scales in phase 1

	Plain lidocaine		*Carbonated lidocaine*	
Premedication	*Pain scale*	*Mean*	*Pain scale*	*Mean*
Lorazepam	1	5.19	1	4.76
	2	6.26	2	5.78
None	1	5.82	1	3.47
	2	6.39	2	5.19

Table VI. Phase 2

	Regular injection	*Deep injection*
Lorazepam		
Yes	45	65
No	14	14
Mean preoperative anxiety score	3.0	3.0
Mean maternal age (yr)	25.8	26.7
Mean gestational age (wk)	8.7	8.5

basis of other subject variables, including maternal age and gestational age.

Results

The pain scales with carbonated lidocaine were significantly lower than the ones with plain lidocaine ($p = 0.036$), but this difference was only 8% (Table I). The pain scales with the deep injections were 25% lower than the scales with the superficial injections ($p = 0.0001$) (Table II). There were no significant differences between the patients who received carbonated lidocaine and the patients who received plain lidocaine in terms of maternal age, gestational age, preoperative anxiety, previous abortions, or whether the patients took lorazepam preoperatively (Table III). There was no significant difference between the pain scales of the patients seen by the three different doctors (Table IV) or by the patients who had taken lorazepam compared with the ones who had not (Table V). There was no significant difference between the patients in phase 2 and phase 1 in terms of gestational age, maternal age, preoperative anxiety, or lorazepam use (Table VI). There was no difference between the pain scales of the patients of the two physicians in phase 2 (Table IV).

Comment

The improvement in pain scales with carbonated lidocaine in phase 1, although statistically significant, is probably not clinically significant or worth the extra cost. The difference with the deep injection is a big improvement clinically and has greatly enhanced pain control. There were no complications of deep injections (such as internal hemorrhage or laceration) during the study period or in the hundreds of procedures done at the clinic with that technique since the study's completion. Glick[9] and Stubblefield[7, 8] have performed thousands of procedures using this technique without incident.

Pain is a difficult parameter to measure, which may account for the lack of previous studies on the pain of abortion procedures or the efficacy of the various local anesthesias and techniques used. Studies on quantification of abortion pain,[5] topical anesthesia for gynecologic procedures,[10] and counseling techniques for therapeutic abortions[11] also used pain scales to measure outcome. Efficacy of techniques is very difficult to evaluate because it is impossible to double-blind and almost impossible to randomize. In my study the conditions in phases 1 and 2 were very similar but not identical. There was no significant difference in the patients' maternal or gestational age. It is unlikely that the physicians improved other aspects of their techniques because they were all experienced physicians. There were no changes in the support staff's protocol or in the physical plant, but it is possible that unrecognized changes occurred other than the one measured. Phase 1 had 10 cm^3 2% lidocaine and phase 2 had 20 cm^3 1% lidocaine; it is possible that this dilution of the anesthetic, rather than just the position of the injection, produced some of the improvement. The magnitude of the difference in pain scales lends validity to the results; it was immediately obvious to the physicians and support staff that deep injection was a better technique. Further research should determine whether the benefit was from the depth of injection or the dilution of anesthetic.

We thank Dr. Lucy Lemieux, Dr. Erle Mitchell, and Christine Tang for assisting with the study, and Dr. Jonathan Berkowitz at the Department of Family Medicine of University of British Columbia for providing statistical evaluation.

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Simultaneous ovarian and intrauterine pregnancy: Case report

Anna Bernabei, MD, Giuseppe Morgante, MD, Massimo Mazzini, MD, Elisabetta Guerrini, MD, Antonio Fava, MD, and Secondo Danero, MD

Siena, Italy

The simultaneous presence of an ovarian and a normal intrauterine pregnancy is a very rare condition. We report such a case seen as an ovarian cyst during gestation. It was immediately and successfully treated, thus allowing for the normal physiologic continuation of the gestation. (AM J OBSTET GYNECOL 1992;167:134-5.)

Key words: Ovarian pregnancy, twin pregnancy, ovarian cyst during gestation

We describe a case of simultaneous ovarian and intrauterine pregnancy.

Case report

A 25-year-old woman, para 0, gravida 1, was hospitalized in our department for an ovarian cyst diagnosed by ultrasonography. The patient was at 9 weeks of gestation, completely asymptomatic. She reported menarche at the age of 14, normal and regular cycles, no allergies, and an appendectomy at age 16.

The gynecologic examination revealed a gravid uterus corresponding to the period of amenorrhea and an orange-sized, mobile mass, localized in the pouch of Douglas. The transabdominal ultrasonographic examination confirmed the presence of a viable intrauterine pregnancy and a 101 × 73 mm ovoid mass with sharp borders in direct contact with the uterine fundus. The inner echo structure was finely granular, hypoechogenic, and characterized by a 10 mm well-defined, small, solid part at the bottom. The ultrasonographic image displayed no characteristics of a cystic gravid corpus luteum.

Other routine examinations were performed (i.e., blood and urine examinations, electrocardiogram, Papanicolaou test, colposcopy, and vaginal bacteriologic examination). All results were within normal limits. The patient was pretreated with one intramuscular injection per day of progesterone for 3 days before surgery.

The woman then underwent laparotomy, with a Pfannenstiel incision, under general anesthesia. At the opening of the abdomen a cystic mass was found in the left pelvis. It was tightly adherent and regular, with a smooth surface. Brownish cystic fluid was aspirated. The cyst was then removed, and a peritoneal washing was performed before suture of the abdominal wall.

The postsurgical period was normal, and the ultrasonographic examinations showed a physiologic progression of the intrauterine gestation. Histologic examination of the cyst showed an ovarian pregnancy.

The patient was discharged 7 days after the operation in good condition. She had a normal gestation until term. A healthy 3.2 kg male baby was delivered spontaneously after a normal labor.

Comment

The incidence of extrauterine pregnancy is one in 200 gestations. The incidence of ovarian pregnancy is one in 34 extrauterine gestations and one in 7000 normal gestations.

The causes of an ovarian implantation are obscure. Numerous hypotheses have been formulated: a delay in the release of the ovum, a thickening of the tunica

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6/1/37252

albuginea, a tubal malfunction, or an intrauterine contraceptive device. Pelvic inflammatory disease, which is often involved in the genesis of a tubal pregnancy, does not seem to be important in the ovarian implantation of the ovum.[1] Ovarian gestation is a rare condition per se, which becomes even less frequent when an intrauterine pregnancy develops.

However, if this unlikely event occurs, we can probably think of a double ovulation with a double fertilization (biovular or bizygotic pregnancy). One embryo can regularly implant in the endometrium, and the other one can abnormally implant in ectopic tissue outside the uterus. The differential diagnosis includes a dermoid cyst, a serous or pseudomucinous cystoma, sometimes a pedunculated myoma or a uterine cornua, and less frequently a malignant tumor.[2] Although ultrasonography can be of some help in the diagnosis, the surgery and the following histologic examination constitute the decisive diagnostic tools.

Surgery is important not only for diagnostic purposes but also as a therapeutic tool. An adnexal mass during gestation must be removed because it can easily rupture or twist or be seen as an intracystic hemorrhage. This, in turn, may cause the obstetrician to suspect an "acute abdomen," thereby jeopardizing the intrauterine pregnancy.

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Antibodies to *Chlamydia trachomatis* in sera of women with recurrent spontaneous abortions

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OBJECTIVE: The relationship between high-titer immunoglobulin G antibodies to *Chlamydia trachomatis* and recurrent spontaneous abortions was evaluated.
STUDY DESIGN: Sera from the female partners of 258 couples with unexplained infertility, no history of chlamydial infection, and negative cervical cultures were diluted 1:128 and tested for immunoglobulin G antibodies to *Chlamydia trachomatis*. A subset of patients was also tested for antibodies to cytomegalovirus, cardiolipin, nuclear antigens, lactoferrin, and spermatozoa.
RESULTS: Seven (41%) of 17 women with three abortions and 6 (60%) of 10 women with four abortions had chlamydial antibodies as opposed to 20 (13.5%) of 148 with no abortions, 6 (12.8%) of 47 with one abortion, and 4 (12.1%) of 33 with two abortions ($p < 0.01$). The incidence of ≥3 spontaneous abortions was 31.8% among women with high-titer chlamydial antibodies and 7.5% among women who had seronegative results ($p < 0.001$). There was no relation between any of the other antibodies and ≥3 abortions or antibodies to *Chlamydia trachomatis*.
CONCLUSION: High-titer immunoglobulin G antibody to *Chlamydia trachomatis* was associated with recurrent spontaneous abortions. The mechanism may involve reactivation of a latent chlamydial infection, endometrial damage from a past chlamydial infection, or an immune response to an epitope shared by a chlamydial and a fetal antigen. (Am J Obstet Gynecol 1992;167:135-9.)

Key words: *Chlamydia trachomatis*, recurrent abortion, antibodies, asymptomatic infection

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Chlamydia trachomatis infections of the female urethra and cervix are often asymptomatic and therefore undetected. The organism can evade host immune defense mechanisms, persist for long periods of time,[1] and ascend to the uterus and fallopian tubes in the absence of clinical symptoms. The sequelae of chronic

Table I. Spontaneous abortions and IgG antibodies to *C. trachomatis* in female partners of infertile couples

No. of abortions	*No. of patients*	*Patients wtih antichlamydia IgG*	
		No.	*%*
0	148	20	13.5
1	47	6	12.8
2	33	4	12.1
3	17	7	41.1*
4	10	6	60.0†
>4	3	1	33.3

Sera from 258 women were tested for IgG antibodies to *C. trachomatis*–infected cells.

*$p = 0.01$.

†$p = 0.006$.

silent chlamydial infections, an increased incidence of tubal infertility, and ectopic pregnancy have been well documented.[2]

The possible influences of a *C. trachomatis* infection on pregnancy outcome are less clear and have recently been reviewed.[3] An association between a cervical chlamydial infection and preterm labor, premature rupture of membranes, or low birth weight have been observed in many, but not in all, investigations.

Most of those studies dealt with women whose chlamydial infections were diagnosed during the second or third trimester of pregnancy. Effects of *C. trachomatis* on the early stages of pregnancy, that is, on the occurrence of first-trimester spontaneous abortions, have been infrequently investigated. Quinn et al.[4] reported an increased prevalence of immunoglobulin (Ig) G antibodies to *C. trachomatis* in women with recurrent spontaneous abortions as compared with fertile controls. The inability of Quinn et al. to detect *C. trachomatis* from the male and female partners by culture prompted their conclusion that a current chlamydial infection was probably not the direct cause of the abortions. A similar possibility was advanced by Rowland et al.[5] in their study of women undergoing in vitro fertilization (IVF). They observed that women with IgG antibodies to *C. trachomatis* who lacked IgM antibodies or positive *Chlamydia* cultures had poorer outcomes after embryo transfer than women who had seronegative results. A subsequent analysis of IVF patients refuted these findings[6]; however, more recent studies have confirmed and extended the original observations.[7,8] Only in those IVF patients whose IgG *Chlamydia* antibody titer was ≥1:128 was there a significantly increased prevalence of spontaneous abortion after embryo transfer.

To further examine the possible relationship between an undetected asymptomatic chlamydial infection and early-stage pregnancy loss, we tested for IgG antibodies to *C. trachomatis* in the female partners of 258 infertile couples and 92 women of proven fertility, all with no history or clinical evidence of chlamydial infection. Our results confirm an association between prior undetected exposure to *C. trachomatis* and increased incidence of recurrent spontaneous abortions.

Material and methods

Subjects. Sera were obtained from the female partners of 258 infertile couples whose cervical cultures were negative for *C. trachomatis*, had no self-reported history of chlamydial infection or pelvic inflammatory disease, and whose tubes appeared to be patent by hysterosalpingography. Sera from 92 women of proven fertility who met these exclusion criteria were also analyzed. A history of infections and pregnancy outcomes was obtained from a questionnaire and an interview.

Antibodies to *C. trachomatis*. Sera were diluted 1:128 in phosphate-buffered saline solution and tested for IgG antibodies to *C. trachomatis* by a commercial assay (ICN Biomedicals, Inc., Costa Mesa, Calif.). In brief, the diluted sera were incubated on slides containing fixed McCoy's cells that had been infected with *C. trachomatis* strain L2. After 45 minutes at 37° C the slides were washed, dried, and incubated with horseradish peroxidase–conjugated rabbit antibody to human IgG. After a second 45-minute incubation the slides were washed, dried, and incubated with a chromogen-substrate solution. After 15 minutes the slides were again washed, dried, covered with a coverslip, and examined microscopically under 10 × 20 magnification. For each sample the entire slide was examined, the presence of IgG antibody to *C. trachomatis* was indicated by the observation of dark blue cytoplasmic inclusions. At least 5% of the cells were positive in the presence of a specific antibody, and a clear distinction between positive and negative sera was easily made. In addition, positive and negative control sera were tested on each slide.

Analysis for other antibodies. Sera were tested for IgG, IgA, and IgM antibodies to spermatozoa by indirect Immunobead binding assay.[9] IgG antibodies to cytomegalovirus were detected by a commerical enzyme-linked immunoassay (Sigma, St. Louis). IgG antinuclear antibody was detected by a commercial indirect immunofluorescence assay with the HEp-2 human cell line fixed to slides (Sigma). IgG and IgM antibodies to cardiolipin and to lactoferrin were detected by enzyme-linked immunoassay, following the procedure of Cowchock et al.[10] with isotype-specific alkaline phosphatase–conjugated goat antibodies to human IgG and IgM (Kirkegaard and Perry, Gaithersburg, Md.). Positive and negative controls were always assayed in parallel to the test samples.

Statistics. Differences in the occurrence of chlamydial antibodies between women with various pregnancy

Table II. Relation between IgG antibodies to *C. trachomatis* and other antibodies in sera of female partners of infertile couples

Antibody	No. positive	
	Chlamydia-antibody positive	*Chlamydia-antibody negative*
Cardiolipin (IgG)	1	3
Cardiolipin (IgM)	5	6
Cytomegalovirus (IgG)	10	10
Nuclear (IgG)	15	8
Lactoferrin (IgG)	2	0
Lactoferrin (IgM)	4	3
Spermatozoa (IgG)	6	5
Spermatozoa (IgA)	2	2
Spermatozoa (IgM)	7	10

Sera from 44 women who had seropositive and 44 women who had seronegative test results for chlamydial IgG were tested for other antibodies by enzyme-linked immunosorbent assay (cardiolipin, cytomegalovirus, lactoferrin), immunofluorescence (nuclear) and immunobead binding (spermatozoa).

histories were evaluated for significance by the χ^2 test with the Yates' correction factor.

Results

Antibodies to *C. trachomatis* in relation to history of spontaneous abortions. Sera were tested for antibodies to *C. trachomatis*–infected cells at a dilution of 1:128. This high titer was chosen to enhance the specificity of the analysis and to focus our attention on those women with vigorous immune responses to *C. trachomatis*. Previous findings indicated that a *C. trachomatis* antibody titer ≥1:128 successfully identified women with an increased incidence of spontaneous abortion.[7] We realize that some women with low-titer IgG antibodies would have remained undetected. Our results, therefore, probably underestimate the incidence of chlamydial antibodies in the populations studied. The use of *Chlamydia*-infected cells allows the detection of antibodies to a wider range of antigens than would be possible in an analysis with only purified elementary bodies. With the present assay immune responses to antigens present on reticulate bodies and elementary bodies and antigens produced by mammalian cells in response to a chlamydial infection can all be evaluated. The assay in its present form does not discriminate between chlamydial antigens and infection-induced host antigens. This may be an important consideration because a stress-induced mammalian protein can cross react with a chlamydial antibody (see Comment).

In our fertile controls 9 (9.8%) of 92 women had IgG antichlamydial antibodies in their sera. Among the 258 patients, 44 (17.1%) had seropositive results. This difference between the two groups was significant ($p < 0.05$). Further analysis revealed, however, that the prevalence of chlamydial antibodies in patients with no history of spontaneous abortions (20 13.5% of 148) or with one (6 12.8% of 47) or two (4 12.1% of 33) prior abortions did not differ from the controls (Table I). Significant increases over the control group in the occurrence of chlamydial IgG antibodies were only observed in women with histories of three ($p = 0.01$) or four ($p = 0.006$) spontaneous abortions. Seven (41.1%) of 17 women with 3 abortions and 6 (60%) of 10 women with four abortions had seropositive results.

Table III. Relation between frequency of spontaneous abortion and maternal age

No. of abortions	*No. of patients*	*Mean age ± SD*
0	148	35.0 ± 4.6
1	47	36.7 ± 5.0
2	32	37.0 ± 5.2
3	17	36.5 ± 4.2
4	10	36.6 ± 5.4

There was a greater than fourfold increase ($p < 0.001$) in the incidence of >3 spontaneous abortions in the 44 (31.8%) women with high-titer chlamydial antibody as opposed to the 214 (7.5%) seronegative women.

Relation between antibodies to *C. trachomatis* and other antibodies. The increased prevalence of IgG antibodies to *C. trachomatis* could have been a consequence of a nonspecific polyclonal activation of B lymphocytes caused by a viral infection[11] or an autoimmune process. Autoantibodies to cardiolipin have been associated with recurrent abortions.[10, 12] The relation between IgG antibodies to *C. trachomatis* and antibodies to cytomegalovirus, cardiolipin, and nuclear antigens and to lactoferrin and spermatozoa was examined in a subset of patients (Table II). The prevalence of any of these antibodies was similar in sera from 44 women who had seropositive and 44 women who had seronegative test results for chlamydial antibodies. In addition, with the exception of chlamydial antibodies there was no relationship between any of the antibodies tested and ≥3 spontaneous abortions.

Relation between maternal age and spontaneous abortions. The incidence of spontaneous abortion may also be related to maternal age; however, in our study no relation between these two variables was noted (Table III).

Comment

Among the female partners of 258 infertile couples and the 92 fertile women with no history or clinical indications of exposure to *C. trachomatis*, IgG antibody reactive with *C. trachomatis*–infected cells was detected in 17.1% and 9.8% of patients and controls, respectively. These findings again highlight the high preva-

lence of asymptomatic chlamydial infections in females. In addition, in confirmation of previous studies those women with histories of three or four spontaneous abortions had the highest prevalence of IgG antibodies to *C. trachomatis*. These observations lead to the conclusion that a chronic silent chlamydial infection may increase susceptibility to first-trimester pregnancy loss, at least in those women with evidence of a vigorous immune response to this organism.

The mechanism whereby a chlamydial infection adversely affects early-stage pregnancy remains to be determined. The continued absence of positive chlamydial cervical cultures in any of our subjects or in their male partners, coupled with similar negative culture findings in the subjects of previous studies,[4,5,8] suggests that it is highly unlikely that viable *C. trachomatis* is present in the majority of subjects whose test results for IgG are seropositive. Our inability to detect IgM antibody to *C. trachomatis* in the subjects whose test results for IgG are positive (unpublished observations) further suggests the absence of an ongoing active chlamydial infection. We cannot exclude the possibility, however, that a nonproductive chlamydial infection, such as has been observed in patients with trachoma,[13] can still be present and can induce a deleterious inflammatory response in the endometrium. Immune system activation and cytokine production in response to chlamydial antigens may interfere with embryo implantation or regulatory mechanisms that protect the fetus from attack by the maternal immune system.

Alternatively, a past chlamydial infection that has subsequently been cleared may have damaged the endometrium sufficiently that successful embryo implantation becomes less likely. There is little evidence to support or refute this possibility.

Another attractive but as yet unproven mechanism involves the induction of an autoimmune response as a result of long-term exposure to *C. trachomatis* antigens. Morrison et al.[14] have demonstrated that a single chlamydial antigen, a 57 kd protein, was responsible for the induction of a hypersensitive immune response in guinea pigs who were previously sensitized to *C. trachomatis*.[14] Furthermore, this protein was shown to be a member of the 60 kd family of heat-shock proteins.[15] Analogous heat-shock proteins are also present in mammalian cells; their production is markedly increased under conditions of stress such as elevated temperature or immune system activation.[16] A member of this protein family exists in man and shares considerable sequence homology with the chlamydial 57 kd protein.[17] The 60 kd heat-shock protein is also one of the first proteins produced by the developing embryo.[18] Therefore sensitization of T lymphocytes to the chlamydial heat-shock protein may result in the generation of lymphocytes that can also react with the analogous human heat-shock protein that can be expressed by the embryo or maternal cells in the pregnant uterus. The resultant immune system activation might directly destroy the embryo or interfere with immune regulatory mechanisms. The detection of cytotoxic T cells specific for epitopes of a mycobacterial heat-shock protein that were also present in the human heat-shock protein and which could lyse human cells expressing the human heat-shock protein has been reported.[19] The presence of antibodies to the chlamydial 57 kd heat-shock protein in patients' sera and expression of the heat-shock protein in infected McCoy's cells is currently under investigation. IgG antibodies reactive with the chlamydial 57 kd heat-shock protein have been detected in some women with *Chlamydia*-associated salpingitis and ectopic pregnancy.[20]

Alternatively, a fetal component that shares an epitope with *C. trachomatis* might have been the eliciting antigen. If true, these women might never have had chlamydial infections, and chlamydial antibodies could merely be a surrogate marker for the presence of an antifetal antibody.

Continued testing for high-titer *Chlamydia* IgG antibody will lead to improved identification and characterization of this subgroup of women whose recurrent abortions or infertility is currently unexplained. We hope that further analysis of these women will clarify the mechanism for their inability to maintain a pregnancy and lead to the development of methods to overcome this problem.

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Randomized, double-blind study of cefotetan and cefoxitin in post–cesarean section endometritis

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OBJECTIVE: The hypothesis of the study is that cefotetan and cefoxitin will be equally efficacious and safe in the treatment of post–cesarean section endometritis.
STUDY DESIGN: In a double-blind, randomized manner 140 patients with post–cesarean section endometritis were treated with cefotetan, 2 gm intravenously every 12 hours, or cefoxitin, 2 gm intravenously every 6 hours. They were followed prospectively for clinical response and side effects. Cure rates between the two groups were compared with the χ^2 test.
RESULTS: The cure rates were 83% for cefotetan and 79% for cefoxitin ($p = 0.56$). No patient required a change in therapy due to adverse effects, and no abnormal bleeding occurred.
CONCLUSION: In this study cefotetan and cefoxitin appeared equally effective in treating endometritis with no difference in side effects or complications. (Am J Obstet Gynecol 1992;167:139-43.)

Key words: Endometritis, cefotetan, cefoxitin

Postpartum endometritis is generally treated with broad-spectrum antibiotics possessing activity against aerobic and anaerobic gram-positive and gram-negative organisms. Cefotetan and cefoxitin are two such antibiotics. Cefoxitin is administered every 6 hours and has activity against *Bacteroides fragilis subspecies fragilis* and the other *B. fragilis* subspecies. Cefotetan is administered every 12 hours and, compared with cefoxitin, has increased activity against gram-negative aerobic bacteria but less activity against the nonfragilis *B. fragilis* subspecies (i.e., *B. ovatus, B. distasonis,* and *B. thetaiotaomicron*).[1] The cefotetan molecule, unlike cefoxitin, contains the methylthiotetrazole group, which has been associated with hypoprothrombinemia during therapy with cefoperazone, cefamandole, cefmetazole, and moxalactam.[2]

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Table I. Outcome of therapy

	Cefotetan (n = 70)	*Cefoxitin (n = 70)*	*Significance*
Evaluable patients	66	63	NS
Cure	55 (83%)	50 (79%)*	NS
Failure	10 (15.1%)†	12 (19%)	NS
Relapse	1 (1.5%)	1 (1.6%)	NS
Days of therapy (mean ± SD)	3.4 ± 1.2	3.2 ± 0.8	NS
Hours to defervescence (mean ± SD)	29.4 ± 18	27.1 ± 21.3	NS
Length of stay (days, mean ± SD)	4.2 ± 2.4	4.3 ± 2.5	NS

NS, Not significant ($p > 0.05$).

*Includes two minor wound infections.

†Includes one wound infection requiring a change in therapy.

Because of its favorable in vitro activity, prolonged half-life, and lower cost, cefotetan has replaced cefoxitin in some hospitals for the treatment of pelvic infections. However, no blinded, randomized study comparing the efficacy of the two drugs has yet been reported.[3-6] Therefore we conducted such a study in 140 consecutive women with post–cesarean section endometritis.

Methods

One hundred forty women who had recently undergone cesarean section were enrolled in this randomized, double-blinded study. The protocol and consent form were reviewed and approved by the Committee Involving Research on Human Beings at the University of Pennsylvania. The standard regimen prescribed for surgical prophylaxis was cefazolin, 1 gm intravenously at cord clamping and then every 8 hours for two more doses, or cefonicid, 1 gm intravenously at cord clamping. Patients with a history of mitral valve prolapse received 2 gm ampicillin plus 80 mg gentamicin at cord clamping and for two more doses postoperatively. Patients were included in the study if the following conditions were present ≥12 hours postoperatively: (1) uterine tenderness, and (2) either a single temperature elevation ≥38.3° C or two temperatures ≥38° C ≥6 hours apart. Patients were not eligible for enrollment if they had another obvious site of infection. Other exclusion criteria were age <18 years, history of an immediate hypersensitivity reaction to a penicillin or cephalosporin, and evidence of antepartum infection (e.g., chorioamnionitis).

Drug regimen. Patients were assigned by a computer-generated randomization schedule to receive either cefotetan, 2 gm intravenously every 12 hours alternating with placebo every 12 hours, or cefoxitin, 2 gm intravenously every 6 hours. All doses were identical in appearance and were dispensed from the pharmacy in 150 ml of 5% dextrose in water. Patients were treated intravenously until deemed cured and were sent home without any oral antibiotics.

Study procedures. The following laboratory tests were performed before the study, every 5 days during therapy, and after the study: complete blood cell count with differential and platelet count, prothrombin time, partial thromboplastin time, liver function tests, serum creatinine, and blood urea nitrogen. Blood and urine cultures were performed in all patients at the time of enrollment. Because of the well-described character of the organisms associated with post–cesarean section endometritis, endometrial cultures were obtained only in patients who failed therapy.[3, 7-10] Patients who did not respond to the study drugs in at least 48 hours were given ampicillin, gentamicin, and either metronidazole or clindamycin. Patients found to have enterococcal bacteremia had therapy changed to ampicillin and gentamicin. Follow-up was obtained 4 to 6 weeks after discharge either at a clinic visit, office visit, or by telephone.

Definitions for evaluating response. Cure was defined as a decrease in temperature and uterine tenderness within the first 48 hours of therapy, with complete resolution of both by the end of therapy. Failure was defined as either a persistently elevated temperature without a trend towards improvement or uterine tenderness without improvement after 48 hours of therapy. All patients with enterococcal bacteremia were considered failures regardless of their clinical responses. Relapses were those patients who met the criteria for cure who subsequently developed signs and symptoms of wound infection, abscess, or recurrent endometritis during the 4 to 6 week follow-up period. All clinical decisions, including those regarding termination of study treatment because of an unsatisfactory response, were made by the primary physicians, not by the study team.

Statistical analysis. Proportions were compared with a χ^2 test or a two-tailed Fisher's exact test. Means were compared with a pooled t test or the Welch's t test in the case of groups with unequal variances.

Results

One hundred forty patients were enrolled in the study; 66 were evaluable in the cefotetan group and 63 in the cefoxitin group. Patients were nonevaluable for the following reasons: chorioamnionitis (four), age <18 years (three), termination of treatment at 24 hours due to rapid clinical response (two), pneumonia (one), and drug administration error (one).

The mean age was 26 years in both groups. Only 13% to 16% of patients had prolonged rupture of mem-

Table II. Episodes of bacteremia

Patient No.	Study antibiotic	Organism	Susceptibility		Clinical outcome
			Cefotetan	Cefoxitin	
1	Cefotetan	*Enterococcus*	R	R	Failure*
2	Cefotetan	*Enterococcus*	R	R	Failure*
3	Cefotetan	*Enterococcus*	R	R	
		Escherichia coli	S	S	Failure*
4	Cefotetan	β-Hemolytic streptococci	S	S	Relapse
5	Cefotetan	*Bacteroides loescheii*	S	S	Cure
6	Cefotetan	*Bacteroides melaninogenicus*	S	S	Failure
7	Cefotetan	Pigmented *Bacteroides sp.*	ND	ND	Cure
8	Cefoxitin	*Enterococcus*	R	R	Failure*
9	Cefoxitin	*E. coli*	S	S	Cure
10	Cefoxitin	*Bacteroides oris*	S	R	
		Peptostreptococcus	S	S	Relapse

R, Resistant; *S,* susceptible; *ND,* not done.
*Failure by definition, organisms resistant to cephalosporins.

branes (>24 hours) or were in labor for >24 hours, with similar percents in both groups. Most patients were primarily cared for by our resident physicians, 86% and 83% in the cefotetan and cefoxitin groups, respectively. Twelve percent and 11%, respectively, had undergone previous cesarean sections.

Complete cure was achieved in 83% (55) of cefotetan patients and 79% (50) of cefoxitin patients ($p = 0.56$) (Table I). The duration of therapy, time to defervescence (<38.5° C), and length of hospital stay were similar in both groups (Table I).

Wound infections developed in one patient treated with cefotetan and in two treated with cefoxitin. The patient treated with cefotetan had persistent fever, and ampicillin, gentamicin, and metronidazole were prescribed afer a bedside debridement to remove a large amount of necrotic tissue. This was considered a cefotetan failure. The cefoxitin patients had minor wound infections and, after bedside debridement, only local care was required. Therefore they were considered cefoxitin cures. All wounds ultimately healed well.

Follow-up was obtained in 85% of patients. One patient in each group had what was considered a relapse during the 4 to 6 week follow-up period. One patient in the cefotetan group, receiving warfarin because of a history of pulmonary embolus, 1 week after discharge fell from a moving car and developed a left lower quadrant hematoma with fever. She declined needle aspiration for culture of the hematoma, and her temperature resolved during a 10 day course of empiric ampicillin, gentamicin, and metronidazole therapy. The cefoxitin patient with relapse was seen in the emergency room with abdominal pain, fundal tenderness, and a temperature of 99.9° F 4 days after discharge from the hospital. She refused readmission and was treated with oral ampicillin and doxycycline; her symptoms resolved.

Table III. Organisms isolated from endometrial cultures (from clinical failures only)

Organism	Total isolates	Cefotetan patients*	Cefoxitin patients
Enterococcus sp.	9	5 (5)	4 (4)
Coagulase-negative staphylococci	5	2 (1)	3 (0)
Diphtheroids	2	1 (1)	1 (0)
Group B streptococci	1	0 (0)	1 (0)
E. coli	1	1 (0)	0 (0)
Enterococcus cloacae	1	0 (0)	1 (1)
B. fragilis group	2	1 (0)	1 (0)
Pigmented *Bacteroides*	1	1 (0)	0 (0)
Bacteroides sp.	1	0 (0)	1 (0)
Fusobacterium	1	1 (0)	0 (0)
Anaerobic gram-positive cocci	1	0 (0)	1 (0)
Candida albicans	2	1 (NA)	1 (NA)

NA, Not available.
*Total number of isolates with number resistant to study drug in parentheses.

Seven patients were bacteremic before initiation of cefotetan therapy, compared with three in the cefoxitin group. Gram-positive, gram-negative, and anaerobic organisms were isolated (Table II). Most organisms were susceptible to both study drugs except for enterococcus, which was cephalosporin-resistant, and *B. oris,* which was cefoxitin resistant. Endometrial cultures from the patients who failed therapy showed mixed aerobic and anaerobic flora. As with the blood isolates, most organisms were susceptible to both cefotetan and cefoxitin. Nine of the 22 treatment failures had enterococci resistant to both drugs isolated from their endometrial cultures (Table III).

Therapy with both drugs was well tolerated. The adverse effects that occurred were generally mild and

Table IV. Adverse effects*

	Cefotetan	*Cefoxitin*
Clinical		
Nausea and vomiting	1 (1.5%)	3 (4.8%)
Diarrhea	6 (9.1%)	4 (6.3%)
Clostridium difficile toxin positive	1 (1.5%)	1 (1.6%)
Vaginal candidiasis	0	1 (1.6%)
Pruritus	0	1 (1.6%)
Headache	1 (1.5%)	0
Laboratory		
Alanine transaminase elevation	0	4 (6.3%)†
Aspartate transaminase elevation	1 (1.5%)	0
Prothrombin time prolongation‡	4 (6.1%)	4 (6.3%)
Mean increase in these patients	1.75 sec	1.45 sec
Hemoglobin drop ≥1.5 gm	4 (6.1%)	5 (7.9%)
Mean drop in these patients	2.0 gm	1.7 gm
Thrombocytosis§	—	—
Mean increase in platelet count (thousands/μl)	127 ± 86	140 ± 84

*Number of patients with percent of patients treated in parentheses.

†1.5 to 2 times normal.

‡Prolongation of ≥1 second or greater than control (12.4 seconds).

§>350,000 per microliter.

self-limited and did not require discontinuation of the study drugs. Clinical adverse effects included diarrhea, nausea and vomiting, pruritus, vaginal candidiasis, and headache (Table IV). Three of the six patients in the cefotetan group who developed diarrhea and one of the four in the cefoxitin group did so 2 to 3 days after changing antibiotic therapy to ampicillin, gentamicin, and metronidazole because of treatment failure. Therefore the relative contribution of the various drugs is uncertain. One patient in each group had *C. difficile* toxin–positive diarrhea. The following laboratory abnormalities occurred in approximately equal numbers in each group: aspartate aminotransferase or alanine aminotransferase levels twice normal, thrombocytosis, anemia, and prolongation of prothrombin time (Table IV).

Comment

Post–cesarean section endometritis was encountered in 40% to 70% of patients before prophylactic antibiotic use became accepted practice.[11] Currently, in spite of routine prophylaxis, endometritis still develops in up to one third of patients in reported series.[11] Risk factors for postoperative infection include young age, low socioeconomic status, extended duration of labor and ruptured membranes, multiple vaginal examinations, and prolonged operation time and delivery because of cephalopelvic disproportion.[11] High rates of cure have been achieved with a number of different antibiotic regimens, all chosen for their efficacy against the mixed aerobic-anaerobic polymicrobial etiology of the infection. Cure rates of 75% to 95% are reported for most with no regimen clearly and consistently superior to the others.[11-16] Therefore issues of cost and ease of administration were important and led us to compare cefoxitin with cefotetan because of the latter's lower cost and less frequent dosing requirement.

The cure rates of 79% and 83% in the present study are similar to our previously reported results comparing cefoxitin with clindamycin plus gentamicin, in which 80% of patients were cured.[17] Some groups have reported higher success rates in treating endometritis. This may be the result of our protocol, which only allowed 48 hours for a clinical response to occur. This cut-off is significantly shorter than what has been used in some studies.[3-5, 9, 18, 19] A second reason for our relatively low response rates may be that we classified patients with enterococcal bacteremia as failures. If we had defined these patients as nonevaluable, the clinical cure rates would have been increased slightly to 87% for the cefotetan group and 81% for the cefoxitin group ($p = 0.31$). In addition, we exercised no influence on the obstetricians' decision to change treatment, and some "failures" may merely represent the care team's impatience with the speed of clinical response. Nonetheless, the double-blinded, randomized nature of our study allows the conclusion that no difference in efficacy could be distinguished between the two drugs.

Our study design elected not to do endometrial cultures unless clinical failure occurred because of the wealth of data previously developed concerning the organisms typically found in post–cesarean section endometritis.[3, 7-10] Therefore it is possible that our two study groups may not have been similar microbiologically. We think this unlikely, owing to the randomized nature of the study and the large number calculated to avoid such statistical aberrations. Moreover, the similarity between the two groups concerning age, incidence and duration of ruptured membranes, duration of labor, incidence of repeat cesarean sections, and socioeconomic status offers further evidence that they were well matched.

Because β-lactam antibiotics containing the methylthiotetrazole side chain have been reported to cause bleeding problems, we monitored patients in both arms of the study for clinical or laboratory evidence of clotting disorders.[1, 2, 20] No abnormal bleeding tendency was detected in this well-nourished population who received only a few days of antibiotic treatment. The mild reduction in hemoglobin and elevation in platelet counts seen in some patients were more likely a result of surgery than of antibiotic treatment. Nonetheless, it seems prudent to be alert for bleeding problems in sicker patients receiving a longer duration of therapy. Other adverse experiences, such as nausea, diarrhea, and abnormal liver enzyme levels, were uncommon and of similar frequency in the two treatment groups.

Cost analysis showed that cefotetan saved an average

of $75 per treatment course. Also, because of the twice daily dosing, nursing time can be used more effectively. Given the equal responses and toxicities, we believe it responsible to use the less costly treatment. Furthermore, we predict that future studies will demonstrate that additional savings can be effected by giving 24 to 48 hours of intravenous treatment followed by a similar period of suitable oral medication.

In conclusion, we found no difference in the clinical cure rate or toxicity of cefotetan and cefoxitin in the treatment of post–cesarean section endometritis in spite of some differences in the chemical configurations and in vitro activities of the two drugs.

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Interactions between intrauterine contraceptive device use and breast-feeding status at time of intrauterine contraceptive device insertion: Analysis of TCu-380A acceptors in developing countries

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OBJECTIVE: This paper is a reassessment of earlier findings from a preliminary analysis of data from a multicenter international trial regimen on breast-feeding and non–breast-feeding women in which events related to insertion, expulsion, and removal of the TCu-380A intrauterine contraceptive device (ParaGard 380) were investigated.
STUDY DESIGN: Performance of the TCu-380A through 12 months after insertion was compared with life-table rate analysis, χ^2, Fisher exact test, or Student's *t* test. Variables were events reported during intrauterine contraceptive device insertion and events throughout the 12 months of study participation by breast-feeding status.
RESULTS: Breast-feeding among intrauterine contraceptive device users was associated with fewer insertion-related complaints and lower removal rates for bleeding and pain. No uterine perforations were reported throughout the study.
CONCLUSION: Differences in the performance of the TCu-380A intrauterine contraceptive device suggest physiologic effects associated with lactational amenorrhea. The TCu-380A intrauterine contraceptive device is a viable option for women breast-feeding at the time of intrauterine contraceptive device insertion. (Am J Obstet Gynecol 1992;167:144-51.)

Key words: Breast-feeding, intrauterine contraceptive device, TCu-380A IUD and breast-feeding, TCu-380A insertion and adverse reactions

The benefits of breast-feeding for both mothers and infants are widely accepted. For the mother it is a natural, convenient, and cost-effective option that offers some protection against pregnancy.[1-6] For the infant prolonged breast-feeding is one of the most important factors for improving chances for survival, growth, and development. In many developing countries breast-feeding also plays a very important role in spacing births.[1, 4] However, current family planning programs in these countries emphasize the use of modern contraceptive methods, most often intrauterine contraceptive devices (IUDs) and hormonal methods (oral pill, implants, and injectables). In some instances lactating women are excluded from using IUDs because of a risk of uterine perforation.[7] On the other hand, IUDs are often recommended for contraceptive use among breast-feeding women because of the lack of adverse effects on lactation.[8]

Family Health International is an international nonprofit organization that conducts research and provides technical assistance in health, family planning, sexually transmitted diseases, and acquired immunodeficiency syndrome. In an earlier analysis of data from Family Health International's multicenter comparative trial regimens of the TCu-380A, breast-feeding women were more likely to experience pain-free insertion, few problems with postinsertion bleeding and pain, no uterine perforations, and a high continuation rate after 6 months of use.[2] Also, breast-feeding women were no more likely to experience accidental pregnancy or intrauterine contraceptive device expulsion than non–breast-feeding women. However, that study represented a preliminary analysis of data from five sites participating in Family Health International's multinational comparative clinical trial regimens of the TCu-380A. More than 20 sites in 11 countries participated in these trial regimens.

Recently Family Health International completed data processing for these sites and is now compiling these data into a worldwide database. The earlier study fo-

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cused on 3- and 6-month rates because follow-up at each of the five sites had not been completed through 12 months. Our article represents a reassessment of these earlier findings with 12-month data from the expanded Family Health International TCu-380A database.

Material and methods

The copper-T-380A (TCu-380A) is a plastic, T-shaped IUD with copper wire wound around the stem and copper collars on each side of the cross arm of the T. This IUD has a total exposed copper surface area of 380 mm^2. It is 36 mm long, 32 mm wide, and has two monofilament marker strings attached to a ball at the bottom of the vertical stem. The TCu-380A has been shown to be an effective contraceptive option, with life-table failure rates of <1 per 100 women after 12 months of use.[10] It has been approved for use in the United States for ≤8 years.

Family Health International's international clinical trial regimens of the TCu-380A IUD were designed to compare this device with the IUD most widely used in each of the participating countries, including the TCu 200, the TCu 220, the Lippes Loop, and the Multiload 250. All sites used identical study protocols and case record forms. IUDs were randomly assigned to participants according to sealed random-allocation envelopes, preprinted before study initiation, and opened at the time of IUD insertion. All study subjects were healthy women who had no contraindications for IUD contraceptive use and who gave informed consent to participate in the study.

The clinical protocol specified that women were to be sexually active and between 18 and 40 years of age. Insertions were performed in the interval period (last pregnancy ended at least 42 days before insertion). Patients were to return for follow-up at 1, 3, 6, and 12 months after insertion and at any time complications occurred. Medical examinations were performed during each contact with the patient. A woman was discontinued from the study if she became pregnant or if her IUD was expelled, displaced, or removed for any reason.

Information on selected sociodemographic characteristics, reproductive and contraceptive histories, and preexisting medical conditions was obtained at the time of admission into the study. The woman's breast-feeding status was determined at admission. Insertion-related events were recorded on the admission form; subsequent pertinent events such as accidental pregnancy, expulsion, removal, complications, and complaints were recorded at each follow-up visit. A total of 2533 women had TCu-380A IUDs inserted at 25 research sites in Nigeria, the Philippines, Mexico, Peru, Cameroon, Pakistan, Sri Lanka, Thailand, Malaysia, El Salvador, and Chile. Insertions were performed between May 1985 and September 1988.

A woman was included in the analysis if her last pregnancy resulted in a vaginally delivered, live, term birth. The study population consisted of 2275 women. At the time of insertion, 1243 of the women indicated they were not breast-feeding and 1032 indicated they were. (Breast-feeding status includes both full lactation and partial lactation, which is defined as providing supplementary food in addition to breast-feeding.)

Performance of the TCu-380A through 12 months after insertion was compared between women who were not breast-feeding and those who were breast-feeding at the time of insertion by life-table rate analysis. Rare but medically serious insertion-related events were compared between the two groups by the χ^2 test of association or the Fisher exact test if the numbers were small. Student's t test was used to compare differences between continuous variables. Gross cumulative life-table event rates were calculated by Potter's method[11] and compared by the log-rank method.[12] Herson's method was used to produce rates adjusted for any differences in baseline characteristics.[13] A p value of <0.05 (two-tailed) was considered statistically significant.

Results

Table I presents data on selected baseline characteristics of the two groups of women. On the average breast-feeding and non–breast-feeding women were similar in their number of live births and in their number desiring additional children sometime in the future. However, non–breast-feeding women were almost 2.5 years older and had completed at least 1 more year of schooling. Non–breast-feeding women were more likely to be using some form of contraception in the month before this TCu-380A IUD insertion, to have had a completed menstrual cycle before insertion, and to have had a shorter time interval (<6 months) between the date the study IUD was inserted and the date their last pregnancy ended (all differences significant at $p < 0.001$).

Events experienced during IUD insertion are presented in Table II. Two failed insertions were reported in this population, with one in each group; these women appear only in Tables I and II. No reports of uterine perforation at the time of insertion were recorded. Other insertion events were rare; when they occurred the number was usually higher among non–breast-feeding women. More non–breast-feeding women than breast-feeding women experienced some degree of pelvic pain during insertion ($p < 0.001$, χ^2 test), and more required cervical dilatation to ease insertion ($p < 0.03$, Fisher exact test). No statistically significant difference were noted between the two groups in the

Table I. Selected baseline characteristics of women using the TCu-38A IUD who were either breast-feeding or not breast-feeding at time of IUD insertion, 1985-1988

Patient characteristics	*Not breast-feeding (n = 1243)*	*Breast-feeding (n = 1032)*	*p Value*
Age (yr; mean ± SD)	28.1 ± 5.6	25.6 ± 5.0	0.001*
Parity (mean ± SD)	2.6 ± 1.9	2.5 ± 2.0	0.786*
Education† (yr; mean ± SD)	9.1 ± 4.6	8.1 ± 4.6	0.001*
Number of additional children desired† (mean ± SD)	0.7 ± 0.9	0.8 ± 0.9	0.206*
Contraceptive used in the month before study?			
None	34.7	79.2	0.001‖
Yes	65.3	20.8	
Menstrual status at insertion§			
Still amenorrheic (%)	8.0	50.6	0.001‖
Resumed menses (%)	92.0	49.4	
Open interval to insertion			
<6 months (%)	19.4	70.0	0.001‖
6-12 months (%)	13.2	17.1	
12-24 months (%)	20.2	10.5	
24+ months (%)	47.2	2.4	

*Student's *t* test.

†Data were unavailable for 131 cases in the not–breast-feeding group and 162 cases in the breast-feeding group.

‡Data were unavailable for 9 cases in the not–breast-feeding group and 8 cases in the breast-feeding group.

§Data were unavailable for 4 cases in the not–breast-feeding group and 1 case in the breast-feeding group.

‖By χ^2.

incidence of cervical laceration at insertion or insertion-related complications such as syncope or diaphoresis. Most of the women were diagnosed with retroverted uteri.

A total of 2207 women (1203 (97%) in the non–breast-feeding group and 1004 (97%) in the breast-feeding group) returned for at least one follow-up visit during the study. Table III presents gross cumulative life-table event rates for 3, 6, and 12 months after insertion. Event rates were found to be comparable for the two groups of women at each follow-up period, with the exception of removals for bleeding or pain. At each time interval studied breast-feeding women experienced fewer removals for this reason than did non–breast-feeding clients.

There were not statistically significant differences in pregnancy and expulsion rates between breast-feeding and non–breast-feeding women. Although breast-feeding women tended to have lower pregnancy rates, pregnancy was very unusual in either group (0.6 per 100 women in the non–breast-feeding group, compared with 0.3 per 100 women in the breast-feeding group). Expulsion tended to be more frequent in breast-feeding women, although expulsion rates were low in both groups as well (2.8 per 100 women among non–breast-feeding women compared with 3.9 in breast-feeding women).

Because the two IUD study groups differed as to (1) age, (2) use of contraception before IUD insertion, (3) menstrual status, (4) length of time between last pregnancy outcome and IUD insertion, and (5) incidence of pain during insertion, life-table termination rates were standardized to calculate adjusted cumulative rates.[13]

Table IV presents 12-month cumulative life-table rates when adjusted for differences in age (<20, 20 to 24, 25 to 29, 30 to 34, 35+ years old); use of contraception before study admission (no, yes); menstrual status at insertion (still amenorrheic, resumed menses); pain at insertion (no, yes); and the length of the open interval between the outcome date of the woman's last pregnancy and the date of IUD insertion (<6 months, 6 to 12 months, 12 to 24 months, 24+ months).

The lack of difference between the two groups for pregnancy, expulsion, or removal for other medical reasons was maintained after the life-table rates were adjusted for differences in each of these baseline characteristics. However, there was a difference in magnitude between the two groups regarding removals resulting from bleeding or pain when life-table rates were adjusted for age.

Breast-feeding women continued to have statistically significantly lower removal rates for bleeding and pain than non–breast-feeding women (5.1 vs 2.9 per 100 women, respectively, $p = 0.015$).

The relationship between the degree of breast-feeding at insertion and the impact on pertinent life-table rates at 3, 6, and 12 months is shown in Table V. Breast-feeding status was separated to differentiate between women breast-feeding and giving supplementary foods (partial) and those breast-feeding without supplementation (full). Women partially and fully breast-feeding experienced similar rates of removal for bleeding or pain, and non–breast-feeding women had a much higher rate of removal for bleeding at 3 months after insertion ($p < 0.001$) and at 6 months after insertion ($p < 0.05$).

Differences among the three groups in bleeding and pain removal rates were no longer statistically significant by 12 months after insertion. Women in the fully breast-feeding group had slightly higher removal rates for bleeding or pain (3.7 per 100 women) than women in the partial breast-feeding group (2.7 per 100 women), but this difference was not statistically significant.

Gross cumulative life-table rates for IUD removals

Table II. Events experienced during IUD insertion for non–breast-feeding and full breast-feeding women using the TCu-380A IUD

Events	*Not breast-feeding (n = 1243)*	*Breast-feeding (n = 1032)*	*p Value*
Uterine position*			0.001†
Anteverted (%)	53.1	49.5	
Retroverted (%)	25.5	35.0	
Midposition (%)	21.4	15.5	
Person performing insertion			0.454†
Obstetrician-gynecologist (%)	75.2	76.6	
Others (%)	24.8	23.4	
Dilation required‡			0.033§
None (%)	98.5	99.5	
Yes (%)	1.5	0.5	
Cervical laceration			0.156§
None (%)	97.2	96.0	
Yes (%)	2.8	4.0	
Pelvic pain			0.001†
None (%)	74.8	82.9	
Yes (%)	25.2	17.1	

*Data were unavailable for 14 cases in the not–breast-feeding group and for 11 cases in the breast-feeding group.
†By χ^2.
‡Data were unavailable for 9 cases in the not–breast-feeding group and for 8 cases in the breast-feeding group.
§Fisher exact test.

because of bleeding and pain for non–breast-feeding women and breast-feeding women who were either amenorrheic or nonamenorrheic at IUD insertion are presented in Table VI. No significant differences in removal rates were detected when comparing rates for non–breast-feeding women and breast-feeding women who had resumed menses, nor were there significant differences when comparing rates for breast-feeding women who had resumed menses with those of women who still had amenorrhea at the time of IUD insertion.

However, significant differences were detected when comparing removal rates at each time period for non–breast-feeding women and breast-feeding women who still had amenorrhea. Overall, breast-feeding women who still had amenorrhea at the time of IUD insertion had the lowest removal rates for bleeding and pain at 3, 6, and 12 months after insertion.

Time since last pregnancy outcome (open interval) was not significantly associated with the incidence of removal of the TCu 380A for bleeding or pain (Table VII). Although women whose last pregnancy ended <6 months before IUD insertion were less likely to have their IUDs removed for bleeding complications than women ≥7 months post partum, differences in the 3-, 6-, and 12-month removal rates were not statistically significant at $p < 0.05$. When bleeding and pain removal rates were adjusted for breast-feeding or menstrual status at the time of IUD insertion, these differences virtually disappeared. Removal of an IUD for bleeding or pain may not be a reflection of postpartum status as much as of whether these women were actively breast-feeding or had amenorrhea (or both) at the time the IUD was inserted.

Comment

The TCu-380A has been shown to provide very effective and safe protection against pregnancy when compared with other IUDs and is becoming the IUD of choice in many family planning programs throughout the developing world.[14] A number of studies have focused on the safety and efficacy of this device, including the effect of IUD use on lactation.[3-6, 9] A limited number of studies have been conducted to assess the impact of breast-feeding status at insertion on the performance of this IUD.

Some studies have found an increased risk of uterine perforation and other complications during IUD insertion in breast-feeding women.[7] However, recent studies have reported few if any uterine perforations among breast-feeding or non–breast-feeding women.[2, 3]

In our study the incidence of accidental pregnancy was found to be low for both study groups. This incidence confirms the results of earlier studies that found the incidence of accidental pregnancy was not affected by breast-feeding status at the time of IUD insertion.[8]

The earlier Family Health International study also did not find an increased risk for expulsion among breast-feeding women.[2] Although expulsion rates were

Table III. Gross cumulative life-table rates per 100 women of pertinent events at 3, 6, and 12 months of use by breast-feeding and non–breast-feeding women inserted with the TCu-380A device, 1985-1988

Pertinent events	*Not breast-feeding (mean ± SEM)*	*Breast-feeding (mean ± SEM)*	*p Value*
Pregnancy (mo)			
3	0.2 ± 0.1	0.0 ± 0.0	NS
6	0.3 ± 0.2	0.1 ± 0.1	NS
12	0.6 ± 0.2	0.3 ± 0.2	NS
Expulsion (mo)			
3	1.7 ± 0.4	2.8 ± 0.5	NS
6	2.2 ± 0.4	3.1 ± 0.5	NS
12	2.8 ± 0.5	3.9 ± 0.7	NS
Bleeding and pain removal (mo)			
3	2.1 ± 0.4	0.3 ± 0.2	0.001
6	3.2 ± 0.5	1.5 ± 0.4	0.05
12	5.1 ± 0.7	2.9 ± 0.6	0.05
Other medical removal (mo)			
3	0.2 ± 0.1	0.5 ± 0.2	NS
6	0.8 ± 0.3	0.8 ± 0.3	NS
12	1.5 ± 0.4	1.4 ± 0.4	NS

Log-rank method was used to determine significance. *NS*, Not significant.

Table IV. Adjusted* cumulative life-table rates per 100 women of pertinent events at 12 months of use by breast-feeding and non–breast-feeding women inserted with the TCu-380A device, 1985-1988

Pertinent events	*Not breast-feeding (mean ± SEM)*	*Breast-feeding (mean ± SEM)*	*p Value*
Pregnancy			
Age	0.6 ± 0.2	0.2 ± 0.2	NS
Menstrual status	0.5 ± 0.2	0.1 ± 0.1	NS
Used contraception	0.7 ± 0.3	0.2 ± 0.1	NS
Open interval	0.7 ± 0.3	0.2 ± 0.1	NS
Pain at insertion	0.6 ± 0.2	0.2 ± 0.2	NS
Expulsion			
Age	3.2 ± 0.6	4.0 ± 0.7	NS
Menstrual status	3.2 ± 0.7	3.9 ± 0.7	NS
Used contraception	3.0 ± 0.6	3.8 ± 0.8	NS
Open interval	3.0 ± 0.6	3.0 ± 0.5	NS
Pain at insertion	2.8 ± 0.5	3.9 ± 0.6	NS
Bleeding and pain removal			
Age	5.1 ± 0.7	2.9 ± 0.6	0.015
Menstrual status	4.3 ± 0.7	3.4 ± 0.7	NS
Used contraception	4.7 ± 0.7	3.5 ± 0.8	NS
Open interval	4.6 ± 0.7	3.7 ± 1.4	NS
Pain at insertion	4.8 ± 0.7	3.3 ± 0.6	NS
Other medical removal			
Age	1.5 ± 0.4	1.4 ± 0.4	NS
Menstrual status	1.3 ± 0.3	1.5 ± 0.5	NS
Used contraception	1.6 ± 0.4	1.3 ± 0.4	NS
Open interval	1.5 ± 0.5	2.2 ± 1.4	NS
Pain at insertion	1.5 ± 0.4	1.3 ± 0.4	NS

Age (<20, 20-24, 25-29, 30-34, 35+); menstrual status (still amenorrheic, resumed menses); used contraception in previous month (no, yes); open interval to insertion (<6 months, 6-12 months, 12-24 months, 24+ months); pelvic pain at insertion (no, yes). *NS*, Not significant.

*Herson's method was used to determine significance.

higher among breast-feeding women at each follow-up interval, the difference in rates were not statistically significant. Breast-feeding women may be at a slightly higher risk of IUD expulsion, but this risk may be more the result of their recent postpartum status than of breast-feeding alone. This risk also held true when these rates were adjusted for differences in the length of time between IUD insertion and the women's last pregnancy outcome.

Removals for bleeding or pain comprise most IUD discontinuations. Breast-feeding women in our study experienced fewer removals for bleeding or pain complications than non–breast-feeding women at each follow-up interval studied. This difference was greatest during the first 3 months after insertion, when the risk of increased bleeding associated with IUD use is highest. Rates were beginning to converge by the end of the study (12 months after insertion), but this difference was still statistically significant.

Breast-feeding status may not be the sole reason for differences in the rates of IUD removal resulting from bleeding and pain complications; breast-feeding women were not similar to non–breast-feeding women across many baseline characteristics. Almost all non–breast-feeding women were menstruating at the time of IUD insertion compared with less than half of the breast-feeding women.

The finding that bleeding and pain removal rates for breast-feeding women with amenorrhea were significantly lower than those removal rates for non–breast-feeding women is not unexpected. Breast-feeding has been found to prolong postpartum amenorrhea (lactational amenorrhea) and to inhibit ovulation and reduce the likelihood that pregnancy may occur.[16, 17] Thus, as long as a woman continues to breast-feed, she is more likely to have amenorrhea and thereby continue to have a lower risk of IUD-related bleeding than menstruating women.

Some of the differences in the baseline characteristics between breast-feeding and non–breast-feeding women, such as use of contraception in the month before IUD insertion, the completion of a menstrual cycle before insertion, and the length of the interval between delivery and IUD insertion are clearly related to breast-feeding status. Therefore it is not a surprise that the differences in bleeding-and-pain removals and in pain at insertion disappear when the two groups (breast-feeding and non–breast-feeding) are adjusted by the above-mentioned differences.

It is important to note that no uterine perforations were reported among these women. It has previously been thought that women who were breast-feeding at

Table V. Gross cumulative life-table rates per 100 women of pertinent 3 and 6 months of use by extent of breast-feeding at the time of insertion of the TCu-380A device, 1985-1988

Pertinent events	*No. of women*			
	Admission	*At 3 mo (mean ± SEM)*	*At 6 mo (mean ± SEM)*	*At 12 mo (mean ± SEM)*
Pregnancy				
Not breast-feeding	1242	0.2 ± 0.1	0.4 ± 0.2	0.6 ± 0.2
Partial	565	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Full	466	0.0 ± 0.0	0.2 ± 0.2	0.6 ± 0.4
Expulsion				
Not breast-feeding	1242	1.7 ± 0.4	2.3 ± 0.4	2.8 ± 0.6
Partial	565	3.3 ± 0.8	3.7 ± 0.8	4.6 ± 0.9
Full	466	2.2 ± 0.7	2.4 ± 0.7	3.0 ± 0.8
Bleeding and pain removal				
Not breast-feeding	1242	2.1 ± 0.4*	3.2 ± 0.5†	4.8 ± 0.6
Partial	565	0.4 ± 0.3	1.6 ± 0.6	3.7 ± 1.0
Full	466	0.2 ± 0.2	1.4 ± 0.6	2.6 ± 0.7
Other medical removal				
Not breast-feeding	1242	0.2 ± 0.1	0.8 ± 0.3	1.6 ± 0.4
Partial	565	0.6 ± 0.3	1.0 ± 0.4	1.7 ± 0.6
Full	466	0.5 ± 0.3	0.7 ± 0.4	1.0 ± 0.5

Partial breast-feeding is defined as breast-feeding with supplementary food given.
*$p < 0.001$ by log-rank method comparing the three feeding groups.
†$p < 0.05$ by log-rank method comparing the three feeding groups.

Table VI. Comparisons of gross cumulative life-table rates per 10 women for removals because of bleeding and pain according to breast-feeding and menstrual status at the time of insertion of the TCu-380A device, 1985-1988

Admission status	*At 3 mo (mean ± SEM)*	*At 6 mo (mean ± SEM)*	*At 12 mo (mean ± SEM)*
Not breast-feeding	2.1 ± 0.4	3.2 ± 0.5	4.8 ± 0.6
Breast-feeding–resumed menses	0.6 ± 0.4	2.1 ± 0.7	3.6 ± 0.9
Not breast-feeding	2.1 ± 0.4*	3.2 ± 0.5†	4.8 ± 0.6‡
Breast-feeding–still amenorrheic	0.0 ± 0.0	0.9 ± 0.4	2.5 ± 0.8
Breast-feeding–resumed menses	0.6 ± 0.4	2.1 ± 0.7	3.6 ± 0.9
Breast-feeding–still amenorrheic	0.0 ± 0.0	0.9 ± 0.4	2.5 ± 0.8

*$p = 0.003$ by log-rank method.
†$p = 0.011$ by log-rank method.
‡$p = 0.038$ by log-rank method.

the time of IUD insertion were at higher risk of uterine perforation than non–breast-feeding women,[7, 17] although other researchers have not always found this to be the case.[9, 18] The absence of reports of uterine perforation in this study cannot be construed to mean that IUD perforation should no longer be considered a risk factor among women using IUDs.

However, these results are reassuring and may suggest that inserters trained under a similar insertion procedure can have low perforation rates. It is also reassuring that no increased risk of perforation in breast-feeding women was observed in a multicenter study involving a large number of women with different individuals performing the IUD insertions.

Of special importance is the lower incidence of pain reported at the time of IUD insertion and fewer removals for bleeding and pain among breast-feeding women, particularly among those women who still had lactational amenorrhea at the time of insertion. The lower incidence of both pain at insertion and pain being a reason for IUD removal is likely to be the result of the same factors that are associated with lactational amenorrhea.

Researchers now accept the fact that an increased secretion of β-endorphin is responsible for the maintenance of lactational amenorrhea. The suckling stimuli produce an increased hypothalamic secretion of β-endorphin, which in turn inhibits the activity of the hypothalamic luteinizing hormone pulse generator.[19] β-endorphin is a potent analgesic, several times more potent than morphine.[20] It has been reported that endorphins increase the threshold of pain in the rat[21]; an

Table VII. Comparisons* of cumulative life-table rates per 100 women for removals because of bleeding and pain by open interval since last pregnancy outcome; gross rates and rates adjusted by breast-feeding and menstrual status at the time of insertion of the TCu-380A device, 1985-1988

Interval status	*At 3 mo (mean ± SEM)*	*At 6 mo (mean ± SEM)*	*At 12 mo (mean ± SEM)*
Gross rates			
Interval ≤6 mo	0.7 ± 0.3	1.8 ± 0.4	3.3 ± 0.6
Interval > 6 mo	2.3 ± 0.4	2.9 ± 0.5	4.7 ± 0.6
Breast-feeding–menstrual status at admission†			
Interval ≤ 6 mo	1.4 ± 0.5	2.7 ± 0.8	3.7 ± 0.8
Interval > 6 mo	1.3 ± 0.3	2.8 ± 0.6	4.1 ± 0.7
Breast-feeding status at admission‡			
Interval ≤ 6 mo	1.3 ± 0.5	2.6 ± 0.7	3.7 ± 0.8
Interval > 6 mo	1.4 ± 0.3	2.9 ± 0.5	4.4 ± 0.6
Menstrual status at admission§			
Interval ≤6 mo	1.0 ± 0.4	2.2 ± 0.6	3.6 ± 0.7
Interval > 6 mo	1.3 ± 0.3	2.8 ± 0.8	4.2 ± 0.7

*By log-rank method; none of the comparisons were found to be statistically significant at $p < 0.05$ at each time interval.
†Defined as non–breast-feeding, breast-feeding–still amenorrheic, breast-feeding–resumed menses.
‡Defined as non–breast-feeding and breast-feeding.
§Defined as amenorrheic and resumed menses.

increased tolerance to pain at the end of pregnancy, attributed to an increase in β-endorphin, has been reported in normal women.[22]

Another hormonal factor associated with lactational amenorrhea is absent or very low estrogen secretion. It has been shown that the sensitivity of the uterus varies according to the phases of the menstrual cycle in the rat.[23] In general, estrogens increase the sensitivity of afferent uterine neural fibers.[25] When rats are in vaginal estrus or proestrus, the hypogastric uterine fibers are more sensitive to stimulation such as pressure or stretching.[24] It is possible that the lower incidence of pain during IUD use among breast-feeding women observed in this study may be because of increased levels of β-endorphin and low levels of estrogens.

These two situations are more likely to be present when breast-feeding women are still in amenorrhea, which is also consistent with the findings of the study. Lower rates of removals for bleeding and pain at 3 and 6 months of IUD use among breast-feeding women who were still in amenorrhea or who were fully breast-feeding at the time of IUD insertion are also consistent with a β-endorphin and estrogen-mediated effect. β-endorphin levels decrease and estrogen levels increase with a reduction in the breast-feeding stimuli, a situation that is likely to occur because the postpartum period is extended.

In summary, these data suggest that removals for bleeding and pain occur within the first few months postinsertion and that women with amenorrhea who are breast-feeding at the time of IUD insertion may be less likely to experience a removal because of bleeding and pain complications during this time than women who are not. An increased risk of adverse effects, including uterine perforation, was not apparent among women using the TCu-380A IUDs who were breast-feeding at the time of insertion. Breast-feeding women experienced easier and less painful insertion procedures and were less likely to prematurely stop IUD use because of bleeding complications associated with IUD use.

Differences in the rate of removal continued to persist over time, suggesting that breast-feeding at the time of IUD insertion may have more impact on long-term continuation of use than was previously expected.[23] What may cause this lower risk is uncertain; most likely it is the result of the amenorrhea of many of the breast-feeding women at the time of IUD insertion and that the women probably continued to have amenorrhea during the early postinsertion months and not specifically to their postpartum status. This effect can only be maintained if women continue to breast-feed and have amenorrhea for a period of time.

Differences detected in the performance of the TCu-380A IUD may be caused by factors associated with lactational amenorrhea and other dissimilarities between breast-feeding and non–breast-feeding women. These findings suggest that use of the TCu-380A is a viable option for women who are breast-feeding at the time of IUD insertion. The potential of a lower incidence of pain at the time of IUD insertion and of lower rates of removals for bleeding and pain can actually be an advantage for the use of IUDs in breast-feeding women, particularly during the period of lactational amenorrhea.

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Survival and disease progression in human immunodeficiency virus–infected women after an index delivery

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OBJECTIVE: Our objective was to provide information on survival and disease progression in human immunodeficiency virus antibody–positive pregnant women undergoing prospective evaluation.
STUDY DESIGN: After an index delivery, 103 human immunodeficiency virus antibody–positive pregnant women were identified and underwent follow-up for 3 years. The patients were assessed medically and/or gynecologically when hospitalized for a human immunodeficiency virus–related illness or at each follow-up visit. The life-table method was used to estimate the cumulative probabilities of survival and remaining free of acquired immunodeficiency syndrome. Cox's proportional-hazards analyses were used to identify prognostic factors for survival and progression to acquired immunodeficiency syndrome.
RESULTS: The majority of human immunodeficiency virus–infected pregnant women were alive 3 years later. Lymphadenopathy syndrome or herpes genitalis was significantly associated with a subsequent diagnosis of acquired immunodeficiency syndrome. Of the 103 original patients, six had acquired immunodeficiency syndrome at the index delivery and acquired immunodeficiency syndrome developed in 24. Approximately 94% of evaluable patients with development of acquired immunodeficiency syndrome had CD4-lymphocyte counts <200/mm³. The most common opportunistic infection was *Pneumocystis carinii* pneumonia. Acquired immunodeficiency syndrome and postpartum zidovudine therapy were independent prognostic factors affecting survival.
CONCLUSION: Survival was affected by Centers for Disease Control group status of human immunodeficiency virus infection at the index delivery. (Am J Obstet Gynecol 1992;167:152-7.)

Key words: Acquired immunodeficiency syndrome (AIDS) in pregnancy, survival of human immunodeficiency virus (HIV)–infected women, Cox's model, time-dependent covariates

The acquired immunodeficiency syndrome (AIDS) had been reported in nearly 19,000 women in the United States as of July 1991.[1] AIDS was the eighth leading cause of death in women 15 to 44 years old in 1987,[2] and in 1990, 11.3% of all new AIDS cases occurred in women.[1] On the basis of current trends, AIDS will be among the five leading causes of death in women of childbearing age in 1991.[2]

Several studies have suggested that women have a lower median survival than men.[3-7] It is unknown whether pregnancy or cofactors such as genitourinary tract infections, sexually transmitted diseases, age[8,9] simultaneous infection with the human T-lymphotropic virus type I (HTLV-I),[10,11] the mode of acquisition of human immunodeficiency virus (HIV) infection,[12] and the biologic properties of HIV variants[13] increase the rate of progression to AIDS. This observational study provides information on survival and disease progression in HIV antibody–positive pregnant women undergoing prospective evaluation after an index delivery.

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Patients and methods

Population characteristics. Between Jan. 28, 1986, and Dec. 21, 1988, 103 HIV antibody–positive pregnant women were identified for clinical follow-up during a pregnancy that resulted in an index preterm or term delivery at Jackson Memorial Hospital. All patients were voluntarily tested for HIV before delivery because of admitted risk factors or medical indications and had either a repeatedly reactive enzyme-linked immunosorbent assay and a confirmatory Western blot or a repeatedly reactive enzyme-linked immunosorbent assay and ultimate development of clinical manifestations of HIV infection as defined by the Centers for Disease Control. Historically, there is a strong correlation between the clinical manifestations of HIV infection and the presence of HIV antibody.[14] The majority of patients were seen for follow-up in a special antepartum clinic. During this period, there were

38,305 deliveries in a predominantly socioeconomically disadvantaged obstetric population ethnically distributed as 45% African-American, 35% Hispanic, 10% Haitian, and 10% white.

In the study population, the majority (53.4%) were Haitian followed by 37 (35.9%) African-Americans, six (5.8%) Hispanics, and five (4.9%) white patients. The mean age at the index delivery was 29.0 years (range 16.5 to 43.5 years). The median length of follow-up after the index delivery was 837 days (range 1 to 2308 days). Most (60.2%) patients were followed up >2 years; 18.4% were monitored from 1 to 2 years, and 21.4% were evaluated for ≤1 year.

Assessments and end points. The defined entry point was an observed pregnancy resulting in a preterm or term delivery. After delivery, the patients were assessed medically and/or gynecologically when hospitalized for an HIV-related illness or at each follow-up clinic visit.

CD4-lymphocyte counts were used to determine the need to treat with zidovudine and to give prophylaxis for *Pneumocystis carinii* pneumonia. Historic and current data on HIV-related medical conditions were obtained by systematic abstracting of the subjects' medical records.

Patients were classified according to the Centers for Disease Control system for HIV infection (Table I) and were not reclassified if clinical findings resolved. AIDS was diagnosed with the revised 1987 Centers for Disease Control surveillance case definition.[15]

Statistical analyses. The outcomes of interest in the analyses were time from entry into the study (index delivery date) to date of: (1) AIDS diagnosis and (2) death (survival time). The cumulative probabilities of survival and remaining AIDS-free after a given number of years were estimated by both the life-table method[16] and Kaplan-Meier analysis,[16] and differences in survivor function among the Centers for Disease Control groups were tested with both the log-rank and generalized Wilcoxon tests. Cox's proportional-hazards analyses[16, 17] identified prognostic factors for survival and progression to AIDS. The outcome variables of primary interest were: the Centers for Disease Control group of HIV infection at the index delivery, a diagnosis of AIDS, and postpartum zidovudine therapy. The covariates included in the models to be controlled were for age, race-ethnicity, and gravidity. The diagnosis of AIDS and postpartum zidovudine therapy were functions of time; therefore they were treated as time-dependent covariates in the proportional-hazards model.[16, 17] Genitourinary tract infections and sexually transmitted diseases (including syphilis, gonorrhea, herpes genitalis, and condyloma) diagnosed during pregnancy, known HIV infection status of the index neonate on the basis of Centers for Disease Control criteria,[18] the occurrence of a subsequent pregnancy, and history of drug use were also tested in the models. The Pearson χ^2 statistic (two-tailed) was used to examine possible associations among the variables. All p values of <0.05 were considered statistically significant.

Table I. Summary of Centers for Disease Control classification system for human immunodeficiency virus infection[14, 15]

Group I	Acute infection
Group II	Asymptomatic infection*
Group III	Persistent generalized lymphadenopathy*
Group IV	Other disease†
Subgroup A	Constitutional disease
Subgroup B	Neurologic disease‡
Subgroup C	Secondary infectious diseases
Category C1	Specified secondary infectious diseases listed in Centers for Disease Control surveillance case definition for AIDS‡
Category C2	Other specified secondary infectious diseases
Subgroup D	Secondary cancers‡
Subgroup E	Other conditions

*Patients in groups II and III may be subclassified on the basis of a laboratory evaluation.

†There is no hierarchy of severity among group IV, subgroups A through E.

‡Includes those patients whose clinical presentation fulfills the definition of AIDS used by the Centers for Disease Control for national reporting.

Results

Impact of HIV infection on maternal outcome. When the Centers for Disease Control classification system (Table II) was applied to the 103 patients at the index delivery, 82 (79.6%) were asymptomatic (group II), 13 (12.6%) had lymphadenopathy syndrome (group III), six (5.8%) had AIDS (group IV, C1), and two (1.9%) had oral candidiasis fulfilling the criteria for other specified secondary infectious diseases (group IV, C2).

Over the long term, of the 82 asymptomatic patients, only 32 (31.1%) did not have progression of HIV during the study. In 22 (21.4%) patients, lymphadenopathy syndrome developed. Of the remaining 28 (26.2%) patients, one died of an unrelated HIV-AIDS cause, AIDS developed in 15, and 12 had progression to constitutional disease (n = 3), other specified secondary infectious diseases (n = 4), and simultaneous constitutional disease and other specified secondary infectious diseases (n = 5).

Regarding the 30 patients with AIDS, six (5.8%) had AIDS at the index delivery and AIDS developed in 24 (23.3%). The most common AIDS diagnosis was *Pneumocystis carinii* pneumonia (11, 36.7%) followed by *Candida* esophagitis and toxoplasmosis (four each) and HIV encephalopathy (three cases). Additional AIDS diag-

Table II. Disease progression of HIV-infected pregnant women

Status at index delivery		*Long-term status*							
Group	*No. of persons*	*Group II*	*Group III*	*Group IVA*	*Group IVB*	*Group IVC1*	*Group IVC2*	*Group IVD*	*Other*
II	82	32	22	3	3	11	4	1	6
III	13		4			7	1		1
IVC1	6					6			
IVC2	2					1	1		

*Other includes six asymptomatic patients, five of whom had simultaneous development of constitutional disease and other specified secondary infectious diseases and one who died of an unrelated HIV-AIDS cause, and one patient who had progression from lymphadenopathy syndrome to AIDS with a mixed infection.

Table III. Cox's proportional-hazards analysis of time until progression to AIDS after index delivery*

Variable	*Hazard ratio*	*95% Confidence interval*	*p Value*
Centers for Disease Control group	4.73	1.79-12.54	0.002
Herpes genitalis	8.05	2.18-29.68	0.002
Age	0.90	0.32-2.51	0.837
Race-ethnicity	1.40	0.59-3.30	0.448
Gravidity	0.57	0.17-1.93	0.362

*All variables were dichotomized: Centers for Disease Control group of HIV infection (II vs III), age (≤30 vs >30 years), race-ethnicity (Haitian vs non-Haitian), and gravidity (0 vs ≥1).

Table IV. Cox's proportional-hazards analysis of survival after index delivery*

Variable	*Hazard ratio*	*95% Confidence interval*	*p Value*
Model I			
Group III	6.08	2.00-18.53	0.00
Group IV	9.68	2.50-38.07	0.00
Age	2.05	0.77-5.49	0.15
Race-ethnicity	1.06	0.40-2.77	0.91
Gravidity	0.40	0.11-1.42	0.16
Zidovudine	0.47	0.09-2.51	0.38
Model II			
AIDS	211.59	48.89-955.50	0.00
Age	2.03	0.71-5.86	0.19
Race-ethnicity	0.88	0.50-2.69	0.83
Gravidity	0.84	0.17-4.07	0.83
Zidovudine	0.14	0.02-1.09	0.06

*Centers for Disease Control groups were coded as three categories (group II was the reference); AIDS was coded as yes or no; age was coded as ≤30 or >30 years; race-ethnicity was coded as Haitian or non-Haitian; gravidity was coded as 0 or ≥1; and zidovudine was coded as used or not used.

noses included one case each of Kaposi's sarcoma, cryptococcosis, cryptosporidiosis, and a mixed infection (*Pneumocystis carinii* pneumonia and toxoplasmosis) and four cases of AIDS, not classified. Twenty-two patients have died, two shortly after the index delivery (i.e., 1 and 5 days), accounting for the wide range of follow-up.

There were no statistically significant differences between the Haitian and African-American subpopulations regarding age, gravidity, known HIV infection status of the index neonate, number of subsequent pregnancies, zidovudine therapy, Centers for Disease Control group status, a diagnosis of AIDS, and death.

Zidovudine therapy was evaluated relative to Centers for Disease Control group status, race-ethnicity (Haitian and non-Haitian), age, and the development of AIDS. No statistically significant differences were noted between recipients and nonrecipients of zidovudine. Patients in whom AIDS did and did not develop were equally likely to have been treated with zidovudine.

Among the 24 with development of AIDS, 17 of 18 (94.4%) evaluable patients had CD4-lymphocyte counts <200/mm³ at the time AIDS was diagnosed. Lymphocyte counts were not a significant predictor of survival.

Progression to AIDS. The Kaplan-Meier estimates of the cumulative probabilities of remaining AIDS-free for patients who were asymptomatic or had lymphadenopathy syndrome on entry into the study are shown in Fig. 1. The cumulative probability of remaining AIDS-free decreased over time in asymptomatic patients from 97.4% ± 1.8% (mean ± SE) at the end of 1 year to 78.8% ± 5.7% 3 years later. For those with lymphadenopathy syndrome, the cumulative probability of remaining AIDS-free was lower at the end of the first year (76.0% ± 12.1%), further declining to 26.6% ± 15.6% 3 years later. The cumulative probability of remaining AIDS-free for asymptomatic patients was statistically significantly greater than for patients with lymphadenopathy syndrome ($p < 0.001$).

The temporal relationship of Centers for Disease Control group status at the index delivery and progression to AIDS was examined with Cox's proportional-hazards model. Patients having lymphadenopa-

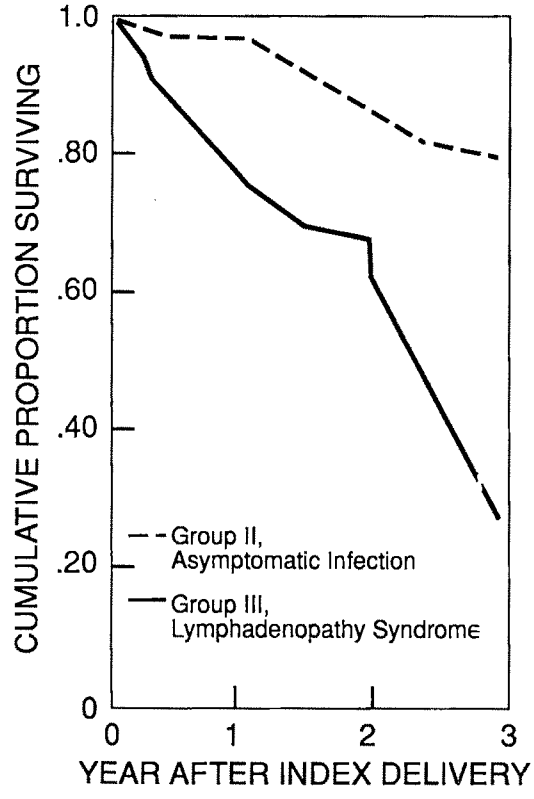


Fig. 1. Three-year cumulative probability of remaining AIDS-free in patients who were asymptomatic (group II) and who had lymphadenopathy syndrome (group III) at index delivery.

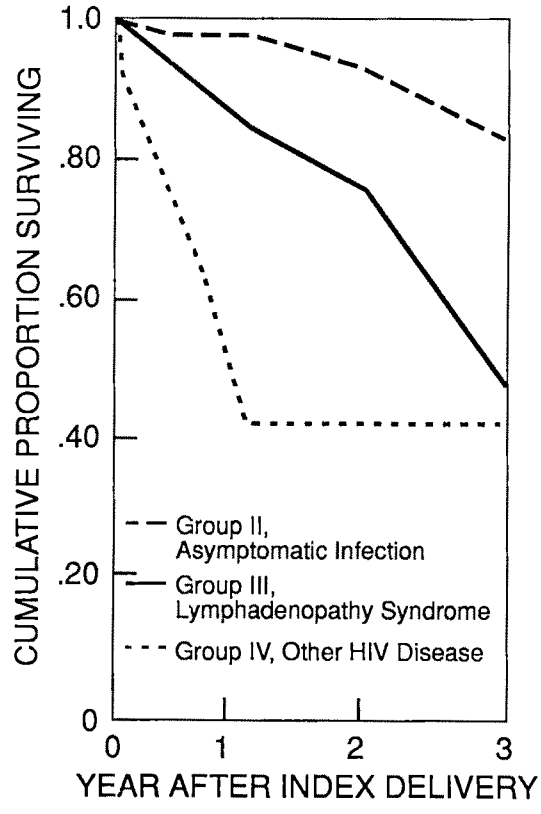


Fig. 2. Three-year cumulative probability of survival in patients who were asymptomatic (group II), had lymphadenopathy syndrome (group III), and had other HIV disease (group IV) at index delivery.

thy syndrome at the index delivery or herpes genitalis during pregnancy were about five and eight times, respectively, more likely to develop AIDS, controlling for age, race-ethnicity, and gravidity (Table III).

Survival of patients. Survival was also examined with Kaplan-Meier analysis. The cumulative probability of survival (mean ± SE) 1 year after the index delivery in asymptomatic patients was 97.4% ± 1.8%, decreasing over time to 83.6% ± 5.2% 3 years later (Fig. 2). For those with lymphadenopathy syndrome, survival was lower at the end of the first year (84.0% ± 10.4%), further declining to 47.5% ± 17.3% 3 years later. Survival in group IV patients was only 42.9% ± 18.7% 1 year after the index delivery. The cumulative probabilities of survival were statistically significantly different among all three groups ($p < 0.0001$). After seven patients who had incomplete follow-up were excluded, the median survival after an AIDS diagnosis was 189 days

The relationship of survival to Centers for Disease Control group status at the index delivery, as well as postpartum zidovudine therapy, was explored with Cox's proportional-hazards model (Table IV, model I). Zidovudine was given to patients at different times when deemed clinically appropriate; therefore this variable was treated as a time-dependent covariate in Cox's model. Group III and IV patients were approximately 6 and 10 times, respectively, more likely to die of AIDS as compared with group II patients, with age, race-ethnicity, gravidity, and postpartum zidovudine therapy controlled for.

The same model was used to explore the relationship of survival to the development of AIDS and postpartum zidovudine therapy with age, race-ethnicity, and gravidity controlled for (Table IV, model II). When these variables were treated as time-dependent covariates, development of AIDS was the most significant predictor of death. Postpartum zidovudine therapy was marginally significant, suggesting its effectiveness in prolonging survival.

Other covariates including genitourinary tract infections, sexually transmitted diseases (including herpes genitalis), known HIV infection status of the index neonate, the occurrence of a subsequent pregnancy, and history of drug use were not statistically significantly associated with survival.

Comment

The majority of HIV antibody–positive pregnant women who were either asymptomatic (group II) or

had lymphadenopathy syndrome (group III) at the index delivery were alive 3 years later. Lymphadenopathy syndrome was a significant prognostic factor for progression to AIDS in this study, in contrast to HIV-infected hemophiliacs[9] and homosexual men,[19] in whom no correlation was found between the presence of lymphadenopathy and progression to AIDS.

The most common AIDS diagnosis in these predominantly Haitian women was *Pneumocystis carinii* pneumonia (36.7%), in contrast to a predominantly non-Hispanic white female population, in whom *Candida* esophagitis was most common (38%).[20] In male HIV-infected patients, *Pneumocystis carinii* pneumonia is the most common AIDS-defining diagnosis.[21] It is possible these discrepant observations are the result of different sample sizes.

The most important laboratory marker for determining the risk of progression to AIDS is the CD4-lymphocyte count.[22, 23] Pregnancy may accelerate the HIV-induced depletion of CD4 lymphocytes and increase the risk of progression to AIDS.[24] Initially, counts were not regularly obtained on all study patients but were ordered on the basis of clinical suspicion that patients might require zidovudine and *Pneumocystis carinii* pneumonia prophylaxis. In 17 of 18 (94.4%) evaluable patients with AIDS, the counts were $<200/mm^3$. Insufficient serial counts were obtained to predict outcome or progression to AIDS, and therefore they were not a significant predictor of survival among the AIDS patients in our study.

There is a paucity of data regarding survival of women with AIDS. Several studies report a lower median survival of women (3.5 to 8.5 months) as compared with men (9.7 to 12.6 months), although the responsible factors are unknown.[3-5, 7] Carpenter et al.,[20] however, reported a longer median survival of 18.5 months in predominantly non-Hispanic white intravenous drug-abusing women, suggesting that ethnic background may be a surrogate marker for socioeconomic status and perhaps enhancing compliance with medical care and improving survival.

In our cohort the median survival after the index delivery was 6.7 months (201 days). As expected, the cumulative probabilities of survival over time decreased within and between all groups and were statistically significantly different. By use of time-dependent covariate methods, the development of AIDS and postpartum zidovudine therapy were predictors of survival.

This study has limitations. First, our cohort did not represent a random sample of all HIV-infected pregnant women. They were screened on the basis of criteria and were not selected from a voluntarily screened population of women. Second, the loss to follow-up was relatively high. It is possible that the patients experiencing progressively worsening HIV-related illnesses were more likely to have follow-up, whereas those remaining healthy were more likely to be lost to follow-up. Thus, we may have overestimated progression and underestimated survival. Consequently, our data should be interpreted with caution. Third, immunologic studies were not obtained from each participant at standardized intervals relative to the index delivery. CD4-lymphocyte counts were not obtained at entry point. Finally, within our clinic population the control group of nonpregnant HIV-infected women was not large enough for study of the impact of pregnancy on HIV infection. Nonpregnant gynecologic patients were not as likely to agree to HIV antibody testing in spite of admitted risk factors as were pregnant women. A prospective controlled cohort study to address this question is presently under way at this institution. In spite of these limitations, our preliminary data reflect current knowledge about survival and disease progression in HIV-infected women after an index pregnancy, including the identification of some prognostic factors. Knowing what these factors are is important, not only in the understanding of the natural history of HIV infection in women but also in the design and analysis of future studies.

In summary, both lymphadenopathy syndrome at the index delivery and herpes genitalis during pregnancy were significantly associated with a subsequent AIDS diagnosis. The most common opportunistic infection was *Pneumocystis carinii* pneumonia. Survival was affected by Centers for Disease Control group status of HIV infection at the index delivery. Our data support a lower median survival of women with AIDS as compared with men with AIDS. It is hoped that ongoing controlled cohort studies will confirm and refine these findings in other populations.

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Enhanced follicle regulatory protein levels accompany reinitiation of ovulatory function after parturition

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OBJECTIVES: We examined the changes in follicle regulatory protein, estrone-3-glucuronide, pregnanediol-3-glucuronide, and luteinizing hormone levels in first-morning urine samples from postpartum, fully breast-feeding women to characterize the reemergence of these urinary hormones after pregnancy ovarian quiescence and early postpartum period and to determine whether follicle regulatory protein could be used prospectively to predict the return of fertility.
STUDY DESIGN: Twenty-five hundred urine samples collected from six postpartum women were evaluated. Daily urine samples collected from normally cycling women were used to establish normal urinary hormone and hormone metabolite cyclicity. Luteinizing hormone, estrone-3-glucuronide, and pregnanediol-3-glucuronide levels were measured by radioimmunoassay. Follicle regulatory protein level was assayed with a double-antibody enzyme-linked immunosorbent assay.
RESULTS: Although follicle regulatory protein levels were found to be very low or undetectable in early postpartum urine, they began to rise with episodes of estrone-3-glucuronide and pregnanediol-3-glucuronide secretion. A χ^2 analysis suggests that increasing urinary follicle regulatory protein levels are most closely associated with the luteal phase of the first menstrual cycles in postpartum women.
CONCLUSIONS: These results suggest that follicle regulatory protein is of little value in predicting either the onset of renewed ovarian activity or the fertile period. (Am J Obstet Gynecol 1992;167:158-62.)

Key words: Follicle regulatory protein, estrone-3-glucuronide, pregnanediol-3-glucuronide, ovulatory function, luteinizing hormone

Although increasing serum levels of estradiol and progesterone are hallmarks of the resumption of cyclic ovarian function after parturition, little is known about ovarian protein secretion during and after the human puerperium. Several ovarian proteins have recently been described and are suspected to be involved in a variety of intraovarian regulations, including follicle development, emergence of the dominant follicle, regulation of the corpus luteum, follicular and corpora luteal gonadotropin interactions, and steroidogenesis.[1] Among these are inhibin, activin, folliculostatin, oxytocin, gonadotropin-releasing hormone–like peptide, and substance P.

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6/1/36674

A new peptide now included in this group, follicle regulatory protein, has been identified in sheep, cow, swine, horse, and human follicular fluid with the inhibition of granulosa aromatase activity as the identifying bioassay.[2-5] The active components of these intraspecies follicular fluid follicle regulatory proteins have similar molecular weights (15,000 d) and isoelectric points (pH 7.4).[6] Follicle regulatory protein has been shown to demonstrate biochemical and biologic properties different from those of growth factors known to influence steroidogenesis, for example insulin-like growth factor-1, insulin-like growth factor-2, epidermal growth factor, transforming growth factor–α, and transforming growth factor–β.[7-9] Follicle regulatory protein is not toxic to granulosa cells in vitro, and its effect on granulosa steroidogenesis is reversible.[10, 11] Follicle regulatory protein has biologic properties different from inhibin or folliculostatin because it does not inhibit the release of follicle-stimulating hormone from rat pituicytes in vitro or in vivo.[12-14]

Levels of follicle regulatory protein were determined from the urine of regularly menstruating women. The nadir of follicle regulatory protein levels occurred during the early and midfollicular phase of the cycle, a time characterized by the largest number of nonatretic follicles. Levels of follicle regulatory protein were

found to rise beginning in the late follicular phase and reached their zenith by the midluteal phase.[15]

However, little is known about the emergence of follicle regulatory protein production by the ovary after the ovary has been quiescent. In our study immunoreactive urinary follicle regulatory protein, estrone-3-glucuronide (EG), pregnanediol-3-glucuronide (PDG), and luteinizing hormone (LH) were measured in consecutive daily urine samples collected from postpartum women throughout the puerperium and the first few ovulatory menstrual cycles to determine when follicle regulatory protein levels begin to rise after parturition. Specifically the investigations were designed to determine whether alterations in urinary follicle regulatory protein levels correspond with the return of follicular maturation and the emergence of the dominant follicle in breast-feeding women after parturition and whether changes in urinary follicle regulatory protein levels during the luteal phase correlated with the typical patterns of urinary steroid excretion.

Material and methods

Patient selection. Daily urine specimens were collected beginning at parturition and continuing for between 170 and 400 days from six women who had previously breast-fed. The women were between the ages of 25 and 30 years, and had been delivered of normal, healthy babies (Apgar scores at 5 minutes >8) at gestational ages between 39 and 42 weeks. First-morning urine samples were collected daily from the parturition through the months of full breast-feeding (i.e., feeding on demand with no other food supplement) until three or four ovulatory menstrual cycles were achieved as documented by regular menses, normal elevations in urinary EG, PDG, and midcycle urinary LH surges.

Hormone assays. Aliquots of 10 ml of the first-morning urine sample were collected and stored at −20° C until urinary creatinine or hormone assays were performed. EG and PDG levels were measured on all samples by direct radioimmunoassay (RIA) by previously published methods.[16-20] Urinary LH level was measured with a double-antibody RIA with reagents (Diagnostic Products Corporation, Los Angeles). Urinary hormones (EG and PDG) were normalized to urinary creatinine levels; creatinine was evaluated spectrophotometrically by the method of Taussky.[21] Follicle regulatory protein was measured by enzyme-linked immunosorbent assay[15] (ELISA) with a dual-epitope approach. Both polyclonal and monoclonal antibodies were raised against follicle regulatory protein with sufficient specificity to allow validation of the ELISA with the sensitivity necessary to quantitate levels of follicle regulatory protein in complex biologic fluids, including urine.[15] A competitive immunoassay was subsequently developed with a murine monoclonal antibody to measure immunoreactive follicle regulatory protein in human urine. Antibodies used in the follicle regulatory protein ELISA do not cross react with transforming growth factor-β, insulin-like growth factors, fibroblast growth factor, LH, follicle-stimulating hormone, prolactin, human chorionic gonadotropin, inhibin, insulin, or transferrin (Table I).[15]

Table I. Cross-reactivity of follicle regulatory protein ELISA with growth factors and reproductive hormones

Sample	*Optical density*
Buffer	0.032
FRP standard (500 ng/ml)	1.010
FRP standard (100 ng/ml)	0.212
Urine pool	0.913
β-TGF	0.040
IGF-1	0.038
IGF-2	0.034
α-FGF	0.041
β-FGF	0.039
EGF	0.046
LH (porcine)	0.036
FSH (porcine)	0.055
Prolactin	0.031
β-hCG	0.041
Insulin	0.045
Transferrin	0.038
Bovine inhibin	0.035

All proteins tested were used at a concentration of 100 ng/ml. Antibody pair used in this validation assay were $7H12F_3H_2$/(2 μg/ml) as the capture antibody, and biotinylated 9D1E1G11/(15 ng/ml) as the detector antibody. *FRP*, Follicle regulatory protein; *TGF*, transforming growth factor; *IGF*, insulin-like growth factor; *FGF*, fibroblast growth factor; *EGF*, epidermal growth factor; *FSH*, follicle stimulating hormone; *hCG*, human chorionic gonadotropin.

Urine from postmenopausal women and women during days 2 through 5 of the menstrual cycle had nondetectable levels of follicle regulatory protein (optical density <0.07). The immunoreactivity of follicle regulatory protein–spiked urine was 100% recoverable and diluted linearly.

Urinary hormone levels in normally cycling women. Urinary levels of EG, PDG, LH, and follicle regulatory protein in normally cycling women are shown in Fig. 1. Hormone levels used as cutoff points for the postpartum women were derived from these data, and were EG > 40 ng/ml urine/mg creatinine; PDG > 1 μg/ml urine per milligram of creatinine; and FRP > 0.07 optical density units. Urinary LH peaks were determined to be ovulatory if peak levels were fivefold the previous baseline measured during the early follicular phase. Moreover, PDG levels after the LH surge were required to be at least 1 μg/ml urine/mg creatinine during the luteal phase for 10 to 12 days to further qualify that cycle to be considered ovulatory.

Data analysis. Urinary steroid metabolites and LH

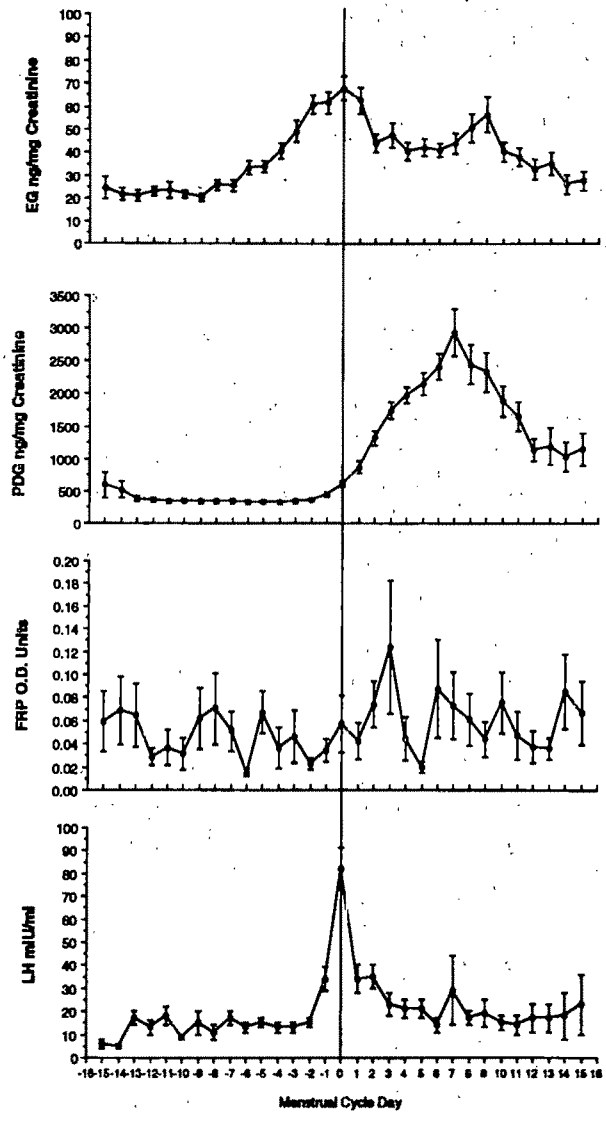


Fig. 1. Mean (±SE) urinary EG, PDG, *FRP*, and LH concentrations. Data normalized to day 0. *Intersecting line*, Day of peak urinary LH EG, PDG, and LH = 60 cycles; *FRP* = 27 cycles. *FRP*, Follicle regulatory protein.

levels are expressed as the ratio of the hormones to follicle regulatory protein (EG/follicle regulatory protein, PDG/follicle regulatory protein, and LH/follicle regulatory protein), and analyzed statistically with a 2×2 χ^2 analysis.

Results

The initial distribution of hormone levels by (1) EG, (2) PDG, and (3) follicle regulatory protein levels are shown in Fig. 2. Five hundred thirty-six urine samples from 26 cycles were found to have EG levels >40 ng/ml/mg creatinine and 931 <40 ng/ml/mg creatinine. When individual urine EG levels were >40 ng/ml/mg creatinine, corresponding PDG levels were

Table II. Follicle regulatory protein–urinary steroid metabolite correlation

Parameters	*Follicle regulatory protein positive/negative (%)*
EG < 40 ng (n = 931)	25
EG > 40 ng (n = 536)	30
PDG < 1 μg (n = 1124)	19
PDG > 1 μg (n = 343)	64*
EG < 40 ng/PDG < 1 μg (n = 836)	22
EG < 40 ng/PDG > 1 μg (n = 95)	67*
EG > 40 ng/PDG < 1 μg (n = 288)	11
EG > 40 ng/PDG > 1 μg (n = 248)	61*

*$p < 0.01$.

Table III. Urine samples collected after reinitiation of folliculogenesis (EDG > 40 ng/ml)

	PDG			
	>1 μg/ml		*<1 μg/ml*	
Subject No.	*FRP positive*	*FRP negative*	*FRP positive*	*FRP negative*
1	12	7	4	15
2	36	8	0	2
3	17	38	4	32
4	7	25	10	78
5	15	41	6	77
6	8	34	5	55
TOTAL	95	153	29	259

FRP, Follicle regulatory protein.

equally distributed as <1 μg/ml/mg creatinine (n = 288) or >1 μg/ml/mg creatinine (n = 248). Follicle regulatory protein values are reported as negative or positive on the basis of previous menstrual cycle data[15] and were found to be negative in most of the urine samples measured regardless of the EG or PDG levels found in the urine. However, the ratio of positive to negative follicle regulatory protein in different groups (Table II) suggests that there is a statistically significant ($p < 0.01$) fourfold to fivefold increased chance of finding elevated follicle regulatory protein levels in urine samples that have elevated PDG (>1 μg/ml/mg creatinine) than for low PDG.

Individual subject distribution for this finding is shown in Table III in urine samples collected after the reinitiation of folliculogenesis, defined as EG levels being >40 ng/ml. Two of the six subjects had elevated follicle regulatory protein levels in the majority of urine samples with high PDG and high EG; four of the six did not. Positive follicle regulatory protein to negative

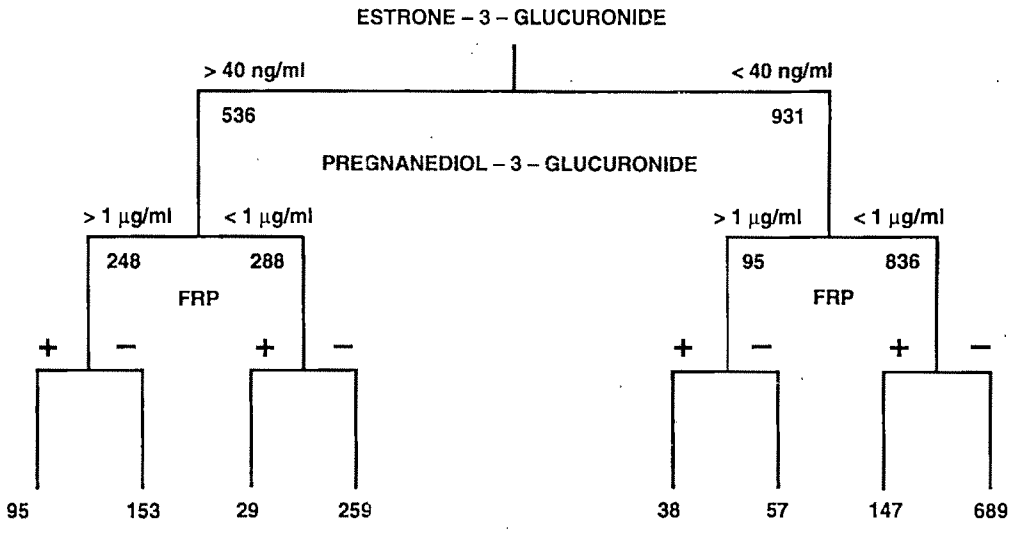


Fig. 2. Distribution of postpartum urine specimens by initial EG, PDG, and *FRP* levels (n = 1467). *FRP*, Follicle regulatory protein.

follicle regulatory protein values were approximately six times higher when PDG levels were elevated (95/153) than when PDG levels were low (29/259).

When the luteal phase was divided into segments comprising the first half (days 14 through 21), middle third (days 18 through 25), and last half (days 21 through 28) in those cycles that had clear LH surges, follicle regulatory protein levels mimicked or paralleled PDG levels in terms of rising and falling concentrations and seemed to be highest in the second half of the cycle in all six subjects (Table IV).

Table IV. Integrated area (arbitrary units) of urinary hormones measured after LH surge (i.e., luteal phase with PDG > 1 μg/ml/mg creatinine)

	Subject No.					
	1	*2*	*3*	*4*	*5*	*6*
No. of cycles	4	5	5	4	4	4
PDG						
First half (days 14-21)	107	162	179	82	108	194
Middle third (days 18-25)	219	244	187	163	142	399
Second half (days 21-28)	240	282	129	120	107	239
Follicle regulatory protein						
First half (days 14-21)	68	95	42	17	9	22
Middle third (days 18-25)	73	98	64	20	16	39
Second half (days 21-28)	79	103	63	21	61	50

Comment

Although follicle regulatory protein has been shown to be a product of the granulosa cell, the physiologic role of this 15,000-molecular-weight peptide has yet to be elucidated. Investigators have speculated that follicle regulatory protein may be involved in the regulation of folliculogenesis, either in the selection of the dominant follicle or possibly retaining smaller follicles in the cohort ready for future ovulation. Because follicle regulatory protein possesses aromatase inhibiting activity, its role in regulating steroid hormone production or steroidogenesis during either the follicular or luteal phase of the menstrual cycle has been suggested.[1, 3-5, 9, 11]

From the data presented here it appears that urinary follicle regulatory protein levels do not predict either imminent fertile cycles or ovulation, per say, in postpartum women returning to full ovarian activity during breast feeding. It does appear that follicle regulatory protein is a marker of the luteal phase and may have a role in corpora luteal function. The close association of elevated follicle regulatory protein levels and high PDG concentrations in the urine may be related in a cause-effect manner, however, verification of this relationship will require further investigation.

The changing pattern of inhibin levels in the normal menstrual cycle have been shown to be quite similar to that of follicle regulatory protein,[12-14] suggesting that these intraovarian peptides may also be regulated in a similar fashion. Other ovarian peptides such as follistatin have been shown to fluctuate during the menstural cycle in ways not dissimilar to follicle regulatory protein and may themselves participate in the regulation of ovarian function. Whether these peptides affect any of the multitude of events that define endocrine physiologic characteristics in the follicular phase of the men-

strual cycle will be shown only by further investigations and improved quantitative assays for these hormones.

Postpartum women offered a unique biologic model for investigating ovarian physiologic characteristics and regulation in that ovarian function has essentially been turned off for a period of many months during gestation and breast-feeding. Ovarian function reemergence as an endocrine organ (either of primary or secondary function) after this quiescent period can provide important insight into the regulation of ovarian hormone production. Our studies suggest that the ovaries of postpartum women produce increasing amounts of follicle regulatory protein in a fashion not unlike other ovarian hormones, particularly steroids and most probably other ovarian peptides. These data further support the contention that follicle regulatory protein is a marker of the corpus luteum rather than of the emerging dominant follicle and may have a primary role in the regulation of corpus luteum formation and function. Whether urinary levels of follicle regulatory protein may be used to predict the return of reproductive function in postpartum, breast-feeding women remains to be shown, although from these studies these levels appear to have a very limited usefulness in this regard. Our data suggest that follicle regulatory protein appears to be temporally associated with the luteal phase of the menstrual cycle in postpartum, breast-feeding women and may be an important marker of luteal phase dysfunctions and pathologic conditions. However, urinary follicle regulatory protein levels often become measurable during the midfollicular phase in women; this measurability should stimulate investigators to look more closely at this intraovarian regulator and its role in folliculogenesis.

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Immunolocalization of the vasoconstrictor endothelin in human endometrium during the menstrual cycle and in umbilical cord at birth

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OBJECTIVE: Our objective was to determine the localization of immunoreactive endothelin in human cyclic endometrium and in umbilical cord during normal delivery and after cesarean section.

STUDY DESIGN: Fixed dated endometrial tissue (n = 41) and umbilical cord (n = 6) were subjected to immunohistochemistry with an antiserum cross reacting with endothelin-1, -2 and -3.

RESULTS: Low levels of stromal endometrial staining were seen throughout the cycle. The strongest staining was in luminal epithelium throughout the secretory phase and in glandular epithelium in the late-secretory phase. In umbilical cord the most intense immunoreactivity was present on the amnion cells on the outer cord, with some staining in intermittent cells in the Wharton's jelly and in umbilical vein cells. No differences were detected between cord from normal delivery or cesarean section.

CONCLUSION: A paracrine role is suggested for endothelin in regulation of endometrial function and a role in vasoconstriction in the umbilical cord at birth. (Am J Obstet Gynecol 1992;167:163-7.)

Key words: Endothelin, endometrium, menstrual cycle, umbilical cord, cesarean section

It is well established that the endometrium is a target tissue for the actions of ovarian estradiol and progesterone, which are responsible for its growth and differentiation in preparation for implantation. More recent is the concept of paracrine and autocrine regulation of endometrial function, and a number of factors acting locally within the endometrium have now been described.[1, 2] It has been postulated that the recently discovered peptide endothelin may also be a paracrine factor in the endometrium.[3] Endothelin was originally identified as a product of endothelial cells, which had a paracrine action on vascular smooth muscle,[4] but it is now also known to be a product of certain types of epithelial cells. Immunoreactive endothelin has been described in epithelial cells of the kidney,[5] rat and mouse trachea,[6, 7] and human lung [8] and in rabbit endometrium.[9] In addition, avascular human amnion cells express endothelin messenger ribonucleic acid.[10]

In the endometrium of immature rabbits, endothelin immunoreactivity is present in the epithelial cells, but is limited to endometrial stromal cells after treatment with sex steroids.[9] Moreover, specific binding sites for endothelin have been demonstrated on both luminal and glandular epithelium of human endometrium.[11]

Umbilical cord is also a potential site of endothelin production. Concentrations of immunoreactive endothelin are increased in umbilical artery and vein and in amniotic fluid at term.[12, 13] The endothelial lining of umbilical veins stains positively with an antibody to endothelin,[14] and these cells release endothelin in culture.[15] Moreover, endothelin is a potent constrictor of umbilical vessels in vitro.[14] What has not been shown is whether the umbilical vein is the only source of endothelin in the cord and whether there are differences between cord at normal delivery and at cesarean section.

This study was therefore designed to determine by immunohistochemistry the localization of endothelin in human endometrium throughout the menstrual cycle of apparently normal women. In addition, the sites of immunoreactive endothelin in umbilical cord samples taken at normal delivery and at cesarean section were defined.

Material and methods

Endometrial tissue was obtained at curettage from 41 women (ages 22 to 43 years) undergoing examination for infertility and with no apparent endometrial dysfunction, who gave their informed consent for collection of the tissue. All tissue collections were approved by the Human Ethics Committee at the Epworth Hos-

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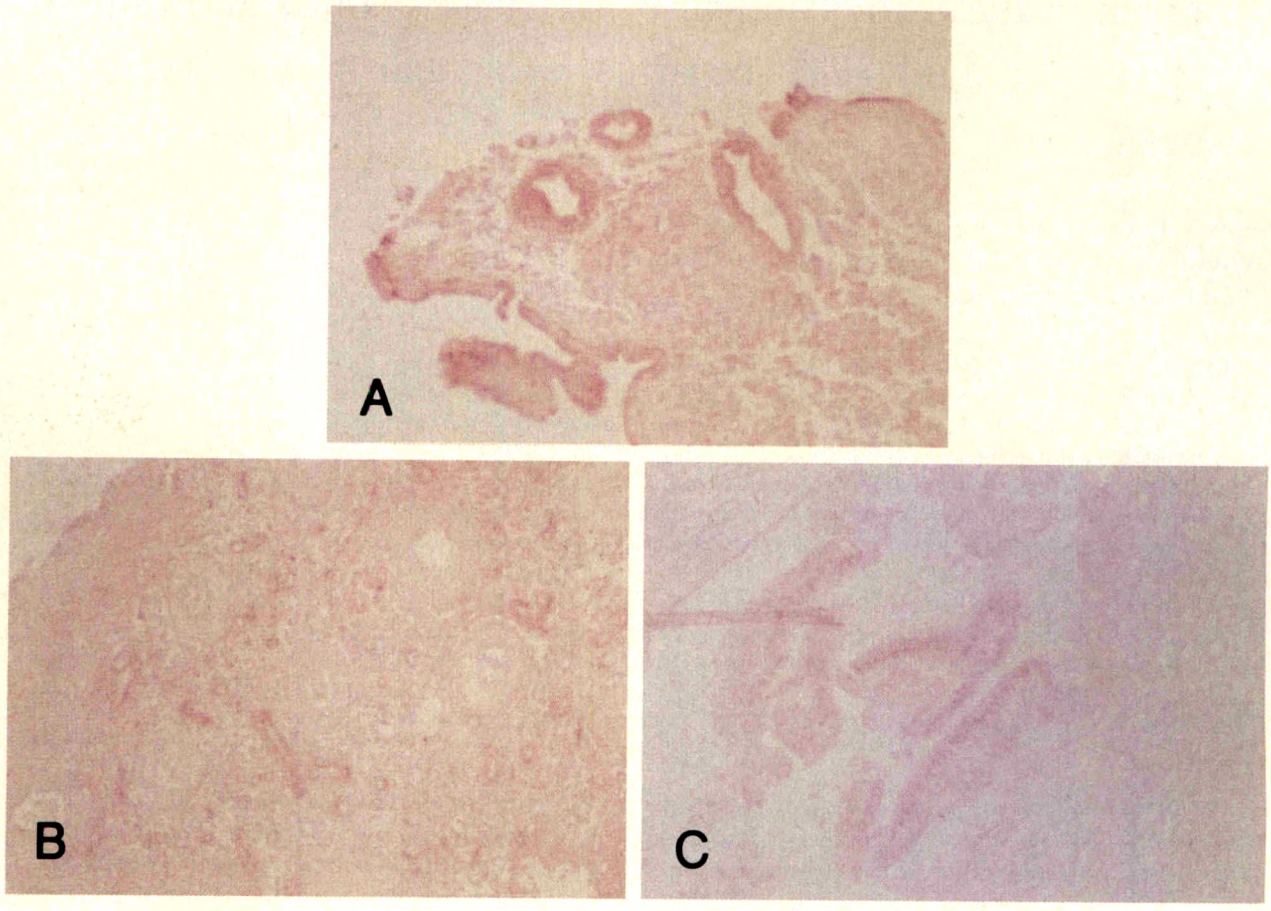


Fig. 1. Immunostaining of endothelin in human endometrium. **A,** Secretory phase endometrium (day 22). **B,** Proliferative phase tissue after incubation with monensin. **C,** Secretory phase endometrial gland after incubation with monensin. (Original magnification: **A** and **B** ×10; **C** ×20.)

Table I. Distribution and relative intensity of positive immunoreactivity for endothelin in human endometrial tissue throughout menstrual cycle

	Menstrual (n = 1)	*Proliferative (n = 9)*	*Early secretory (n = 9)*	*Midsecretory (n = 16)*	*Late secretory (n = 6)*
Glandular epithelium	1	0	1	1	2
Luminal epithelium	NA	0-1	2	2	2
Stroma	1	1	1	1	1

NA, Not available. Intensity of staining in each compartment was scored as negative *(0)*, low *(1)*, moderate *(2)*, or intense *(3)*, related to a positive control sample repeated in all runs. One section from each sample was scored after comparison with its own negative control. Within each group, mean intensity was quantitated by adding the scores and dividing by the number of sections.

pital. A small piece of tissue was taken for routine pathologic studies and endometrial dating, and the rest was immersed in phosphate-buffered formalin (pH 7.4) for 16 to 18 hours before washing in phosphate-buffered saline solution and embedding in wax. Tissues were allocated to four treatment groups: proliferative ($n = 9$), early secretory ($n = 9$), midsecretory ($n = 16$), and late secretory ($n = 6$). In addition, one menstrual stage sample was obtained. Some tissues were divided in two, one half being fixed immediately and the other incubated for 16 hours at 37° C in medium 199 (Flow Laboratories, North Ryde, Australia) containing 5 μmol/L monensin (Sigma Chemical Co., St Louis) before fixation.

Umbilical cord was obtained either at normal, term vaginal delivery ($n = 3$) or at cesarean section ($n = 3$) and fixed similarly.

A polyclonal antiserum against endothelin was obtained from Cambridge Research Biochemicals (Northwich, England) and used at a dilution of 1:2000. This antiserum was developed against endothelin-1 but cross reacts with both endothelin-2 and endothelin-3. Anti-

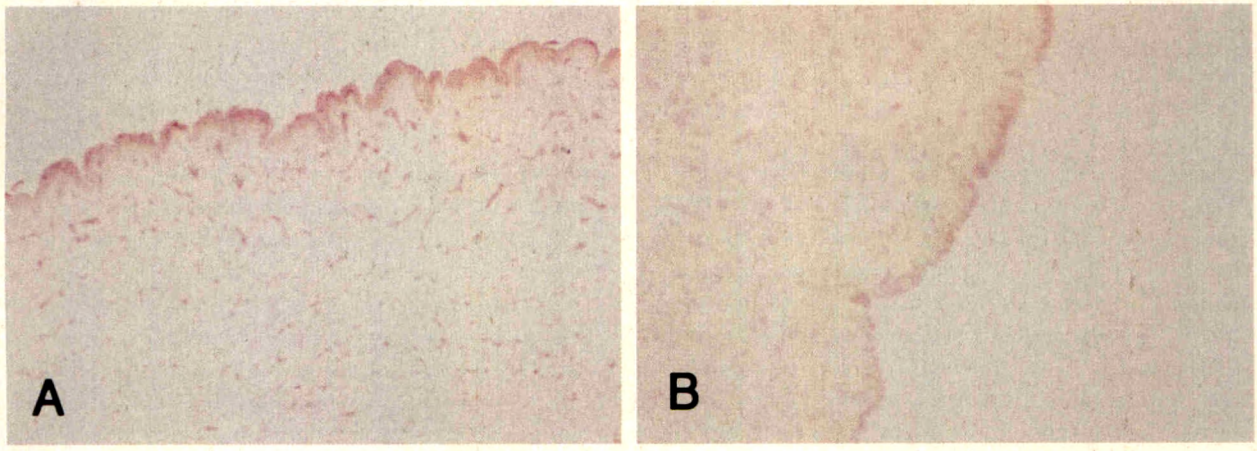


Fig. 2. Immunostaining of endothelin in umbilical cord. **A,** Outer surface of cord showing positive immunostaining for endothelin in amnion cells and in intermittent cells in Wharton's jelly. **B,** Positive staining for endothelin in endothelial cells in umbilical vein. (Original magnification × 10.)

serum against leukocyte common antigen was purchased from Dakopatts A/S, Glostrup, Denmark, and used at a final dilution of 1:100.

Samples were randomized for processing in batches with a section from a standard secretory phase endometrial sample being included with each batch as a positive control and for confirmation of reproducibility between batches. Three separate sections from each sample were processed, one with primary antiserum, one with normal rabbit serum (at the same dilution as the primary antiserum) and one separately stained with hematoxylin and eosin to establish morphologic features. Some additional samples were treated with antiserum that had been preincubated with an excess of endothelin-1 (Peninsula Laboratories, Belmont, Calif.). Immunohistochemical examination used the Strep-AviGen supersensitive kit (Bio Genex Laboratories, San Ramon, Calif.) with the supersensitive alkaline phosphatase system with fast red chromagen substrate and 0.65 mg/ml levamisole (Sigma). Reaction with primary antiserum or normal rabbit serum was for 30 minutes at 22° C. Stained sections were mounted in aqueous mountant and examined under an Olympus (Tokyo) BH2 microscope by two observers. Intensity of positive staining was given a score of 0 (negative), 1 (low), 2 (moderate), or 3 (strongly positive) for each cell type within each section. In a small number of sections (8%) luminal epithelium was not available for scoring. Mean scores within a treatment group were obtained by adding individual scores and dividing by the number of sections.

Results

Endometrial samples. Positive immunostaining for endothelin was observed in all endometrial tissues and is summarized in Table I. Low levels of immunoreactivity were observed in endometrial stroma in all tissues; this did not vary with the stage of the menstrual cycle, although there was considerable variation between individuals. The strongest staining was seen in luminal epithelium throughout the secretory phase but little staining was seen in this compartment during the proliferative phase. No immunoreactive endothelin was detected in glandular epithelium in the proliferative phase, but it was present throughout the secretory phase with the greatest intensity of staining occurring late in the cycle (Fig. 1, *A*). Staining was absent in sections treated with normal rabbit serum in place of antiserum or when antiserum was preadsorbed with endothelin-1. After treatment with monensin, there was a marked accumulation of immunoreactive endothelin in some of the cells, particularly in endothelial cells (Fig. 1, *B*) and in the glandular epithelium in secretory phase samples (Fig. 1, *C*). In the control secretory phase tissue processed in every batch, staining was very consistent throughout the period of the study. Regional differences within any individual tissues were small and less than differences between tissues within a group.

Umbilical cord. In sections from all subjects the most intense immunostaining for endothelin was apparent in the amnion cells on the outer surface of the cord (Fig. 2, *A*). This was consistent in all samples examined. In addition, there was staining of intermittent cells in the Wharton's jelly below the amnion (Fig. 2, *A*). These cells have not been positively identified, but it is unlikely that they are cells of hemopoietic origin, because they did not stain positively with antiserum to leukocyte common antigen. In addition, endothelial cells lining the umbilical vein stained positively for endothelin in five of six samples (Fig. 2, *B*), and in one sample some staining was apparent in cells lining the umbilical artery. No differences were observed between samples of cord obtained at normal delivery or at cesarean section.

Comment

The presence of immunoreactive endothelin has been demonstrated in both stromal and epithelial cells of the human endometrium. The cyclic nature of its localization in both luminal and glandular epithelium suggests differential regulation, possibly by ovarian steroids, in these cells but not in the stromal cells. These studies confirm and extend the preliminary findings of van Papendorp et al.,[16] who described immunoreactive endothelin localized to endometrial glands and most intense at the endometrial-myometrial junction. In their study all gland cells were said to be stained in secretory tissue, but there was a more heterogeneous picture in the proliferative phase. However, they did not describe staining in the endometrial stroma, this being a consistent finding in the large number of tissues in our study. Differences between the studies may be explained by the much longer delay between tissue collection and fixation for hysterectomy samples and those collected by curettage. In a detailed study with rabbit uterus, Maggi et al.[9] showed intense immunoreactivity for endothelin in the endometrial epithelium of immature rabbits. After treatment with 17β-estradiol, either with or without progesterone, endothelin was detected only in the uterine stroma. This contrasts with the situation described here in human endometrium, which suggests that progesterone administration after estrogen priming is necessary for the expression of endothelin in the epithelial cells.

To our knowledge the only other reported immunolocalization of endothelin in the umbilical cord is that of Haegerstraand et al.,[14] who demonstrated positive staining in the endothelial lining of umbilical veins. As in the current study staining was weak in umbilical arteries. However, they did not examine other parts of the cord, although they suggested that other origins of umbilical blood endothelin should be considered. Given the relative intensities of immunoreactive endothelin in amnion cells and in endothelial cells, we would suggest the former as a major source of endothelin in the cord. The nature of the other cells in the Wharton's jelly that contain immunoreactive endothelin remains unknown. We originally postulated that they may be macrophages because messenger ribonucleic acid for endothelin-1 has been demonstrated in macrophages.[17] However, these cells did not show positive immunostaining with the antibody to leukocyte common antigen. Wharton's jelly, a mucomyxoid tissue rich in mucopolysaccharides in which are embedded collagen fibers, mast cells, and large flat stellate cells, has as its known function mechanical protection of the umbilical vessels from trauma. However, the tissue is metabolically very active and may well have other important functions.

No differences in immunoreactive endothelin were demonstrated between cord obtained at normal delivery or at cesarean section; this is consistent with data that neonates delivered by elective cesarean section had similar endothelin levels in blood and amniotic fluid as that measured after normal delivery.[13] A possible function for endothelin at birth may be a contribution to the fetal hemodynamic changes. Indeed, human umbilical vein smooth muscle cells possess specific binding sites for endothelin,[18] and endothelin is a potent constrictor of umbilical vessels in vitro.[14]

The finding of endothelin-like immunoreactivity in cells does not necessarily imply synthesis of the peptide. Alternative explanations are uptake by the epithelial cells or cross-reactivity of antibodies with similar epitopes in other as yet undefined molecules. In cultured endothelial cells endothelin, like many other peptide hormones, is specifically localized in the rough endoplasmic reticulum, Golgi cisternae, and Golgi vesicles.[19] Therefore it was expected that if processing is by the same route in the endometrium, incubation of endometrial tissue with the ionophore monensin (which blocks intracellular transport of protein at the level of the Golgi apparatus[20]) would cause accumulation of endothelin in the cells in which it is synthesized. Demonstration of such accumulation in epithelial cells supports the supposition that endothelin is indeed synthesized in these cells. Production of endothelin by endometrial cells has recently been measured (Salamonsen LA. Unpublished observations) but the presence of specific messenger ribonucleic acid in endometrial tissue remains to be demonstrated.

Other questions of fundamental importance also remain to be answered. It has not yet been demonstrated whether there is any structural or functional difference between endothelin of epithelial compared with that of endothelial cell origin. If endothelin is produced by endometrial cells, does it act locally and on which cells? Biologically active receptors for endothelin are present on rabbit myometrium, these being increased by estrogen and decreased by progesterone,[9] but endometrial binding was not examined. In the human endothelin promotes contraction frequency in myometrium[21] and in the endometrium specific binding sites are present on glandular epithelium and on vascular endothelium at the endometrial-myometrial junction.[11] It has recently been reported[22] that endothelin increased prostaglandin release from explants of human endometrium, although this action requires confirmation. Thus the possibility exists for paracrine activity of endothelin in the uterus. A number of actions have now been described for endothelin in addition to its originally described function of vasoconstriction. These include mitogenic actions on smooth muscle,[23] bronchoconstriction,[24] and modulation of autonomic transmission.[25] Possible uterine functions include regulation of endometrial bleeding, vasoactivity associated with implan-

tation, mitogenesis, or regulation of secretion of other biologically active molecules.

In conclusion, immunoreactive endothelin has been localized in both human endometrium and umbilical cord. Accumulation of immunoreactivity after incubation of endometrial tissue with monensin suggests local synthesis of the peptide. An understanding of the means by which endothelin synthesis is regulated and of its local functions awaits further studies.

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Reduced platelet tritium-labeled imipramine binding sites in women with premenstrual syndrome

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OBJECTIVE: We studied the possible role of serotonergic systems in the cause of premenstrual affective symptoms.

STUDY DESIGN: The binding of tritium-labeled imipramine to platelets is thought to parallel central nervous system binding and to indicate serotonergic activity. We measured platelet tritium-labeled imipramine binding sites in the follicular and luteal phases in 12 controls and in 9 women with well-documented late luteal phase dysphoric disorder. In statistical analyses we used repeated measures analysis of variance, with Student–Newman-Keuls and Duncan's one-tailed *t* tests, and Pearson's *r*.

RESULTS: The values of subjects with late luteal phase dysphoric disorder were lower than those of controls (F [1,39] = 5.13, $p = 0.03$). Both follicular and luteal phase levels were lower in subjects with late luteal phase dysphoric disorder but reached statistical significance only in the follicular phase.

CONCLUSION: Lower platelet tritium-labeled imipramine binding in women with late luteal phase dysphoric disorder supports the hypothesis that alteration of central serotonergic systems may contribute to premenstrual dysphoric symptoms. (Am J Obstet Gynecol 1992;167:168-72.)

Key words: Late luteal phase dysphoric disorder, premenstrual syndrome, depression, tritium-labeled imiprimine binding

The premenstrual occurrence of a variety of physical and emotional symptoms has been termed premenstrual syndrome (PMS) or, when affective symptoms are emphasized, late luteal phase dysphoric disorder.[1] Although epidemiologic and clinical evidence demonstrate significant psychiatric comorbidity in PMS and late luteal phase dysphoric disorder,[2] studies of putative biochemical markers of depression such as the dexamethasone suppression test[3] and the thyrotropin-releasing hormone stimulation test[4] have not revealed consistent deviations from normal values in women with PMS.

Platelet tritium-labeled imiprimine binding sites, which are believed to represent receptor sites that label the presynaptic serotonin transporter on the presynaptic nerve terminal, are lower in number in drug-free depressed patients during acute depressive episodes than they are in controls.[5] There is some evidence that the number of these sites returns to normal several months after clinical remission of depression, response to psychotropic medication,[6] or electroconvulsive therapy.[7] This platelet binding site has, therefore, been described as a state marker.[8] Because the affective state changes in a predictable fashion in late luteal phase dysphoric disorder, this disorder provides a model for the investigation of platelet [^{3}H]imipramine binding sites and their association with depression.

This study investigated the number and affinity of platelet [^{3}H]imipramine binding sites across the menstrual cycle in women without clinical affective disorder who fulfilled criteria for late luteal phase dysphoric disorder.

Material and methods

Twenty-three women aged 24 to 42 (mean 32.2 ± 6.0 SD) were recruited by advertisement and word of mouth to participate in a study of cardiovascular and neuroendocrine reactivity across the menstrual cycle. Subjects with late luteal phase dysphoric disorder and controls were of equivalent parity (mean ± SD 0.63 ± 0.98 for all subjects, range 0 to 2), and all were within ± 15% of ideal body weight. Women with late luteal phase dysphoric disorder reported symptoms for 3 to 11 years (mean ± SD 5.7 ± 2.8) and participated in the study primarily to obtain free evaluation for their premenstrual symptoms. Subjects gave informed consent under a protocol approved by the

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institutional review board and were paid for their participation.

All subjects completed the Premenstrual Assessment Form[9] as a screening instrument and then completed prospective symptom charting over two consecutive menstrual cycles. Twelve women were designated asymptomatic controls, and nine met the DSM-IIIR criteria[1] for late luteal phase dysphoric disorder. A symptom was scored as present and cyclic if it was rated at ≤2 on a six-point ordinal scale throughout the postmenstrual follicular phase of the menstrual cycle and was present at moderate or severe levels (a score of ≥4) during at least 4 days of the premenstrual week. During baseline cycles, ovulation was detected by basal body temperature recordings. During cycles in which laboratory sessions took place, ovulation was documented by the Q-test (Becton Dickinson, Franklin Lakes, N.J.).

A total of 142 subjects were screened. All subjects met the following additional inclusion criteria: a normal Minnesota Multiphasic Personality Inventory profile, which was completed during the follicular phase; absence of Axis I diagnosis on the Structured Clinical Interview for the DSM-III-R[10]; a normal follicular phase Beck Depression Inventory score[11]; the absence of serious medical illness such as thyroid dysfunction; ingestion of no psychotropic, hormonal, or other medications; and a menstrual cycle length of 24 to 32 days. All subjects had normal complete physical and gynecologic examinations and normal laboratory studies, including complete blood counts, urinalysis, and thyroid-stimulating hormone and free thyroxine evaluations.

Subjects completed two laboratory sessions in the follicular and luteal phases of the menstrual cycle in counterbalanced order. Relative to the day of ovulation, cycle days of the laboratory studies were similar for control subjects and those with late luteal phase dysphoric disorder (follicular: control -5.1 ± 1.8 vs late luteal phase dysphoric disorder -6.2 ± 2.2, NS; Luteal: control 9.6 ± 2.4 vs late luteal phase dysphoric disorder 8.7 ± 1.5, NS). All sessions began between 1:30 and 3 PM. Each session lasted approximately 75 minutes, during which subjects were exposed to various stressors. At the beginning and end of each session, 35 ml of blood was drawn into evacuated glass tubes containing 2 ml of acid citrate-dextrose solution, which served as an anticoagulant. Platelet-rich plasma was obtained by centrifugation at 100*g* for 2 minutes; centrifugation was repeated three times. The platelet-rich plasma was then centrifuged (16,000*g* for 10 minutes at 4° C), and the resulting platelet-rich pellet was suspended in 50 mmol of Tris buffer (pH 7.5 at 4° C) containing 150 mmol of sodium chloride and 20 mmol of ethylenediaminetetraacetic acid by means of a Polytron homogenization medium probe (Polytron, Brinkmann Instruments, Westbury, N.Y.) at setting of 4 for 10 to 15 seconds. After centrifugation at 39,000*g* for 10 minutes, membranes were prepared by hypotonic lysis in 5 mmol Tris (pH 7.5), containing 5 mmol ethylenediaminetetraacetic acid and were then homogenized and centrifuged at 39,000*g* for 10 minutes. The supernatant was discarded, and the pellet was washed by resuspension in 70 mmol Tris (pH 7.5) and was centrifuged again at 39,000*g* for 10 minutes. The washed pellet was finally resuspended in the assay buffer, which consisted of 50 mmol Tris (pH 7.5 at 4° C) containing 120 mmol of sodium chloride and 5 mmol of potassium chloride. Aliquots were taken for protein determination. Platelets were then frozen at 70° C until they were used for the binding assay.

Platelet [3H]imipramine binding was conducted with a modification of the procedure of Nemeroff et al.[12] In each sample, total binding was assayed at seven different concentrations (0.25 nmol to 8 nmol) of [3H]imipramine (specific activity 45.4 Ci/mmol); the dissociation constant (K_d) is approximately 1 nmol. Each assay tube (all concentrations were measured in triplicate) contained approximately 100 μg of platelet protein, and the final assay volume was 250 mm^3. Tubes were incubated with the radiolabel for 60 minutes on ice. The reaction was terminated by addition of 5 ml of ice-cold assay buffer, and bound radioactivity was collected by filtration under vacuum over glass fiber filters presoaked for 3 hours in buffer. The filters were then washed three times with 5 ml of ice-cold assay buffer, and bound radioactivity was collected by filtration under vacuum over glass fiber filters presoaked for 3 hours in buffer. The filters were then washed three times with 5 ml of ice-cold assay buffer. Nonspecific binding was defined as binding persisting in the presence of 100 μmol of desipramine. Specific binding represented 80% of total binding, which was less than 5% of the total counts added. Scatchard analysis was used to obtain the maximum binding and K_d for [3H]imipramine. Linear regression of Scatchard plots was used to determine the correlation coefficient *r*. Assays with $r < 0.90$ were excluded. The interassay coefficients of variation were 10% for maximum binding and 5% for K_d.

Comparison of the first and second assays of imipramine binding sites in each laboratory session was performed by paired Student *t* test. The Student–Newman-Keuls test was used to compare pooled [3H]imipramine level results from controls with those from subjects with late luteal phase dysphoric disorder. Repeated measures analysis of variance was used to measure group (control vs late luteal phase dysphoric disorder) × time (follicular vs luteal) interactions. Analyses of variance with Duncan's one-tailed *t* tests were used to compare results from both groups within one

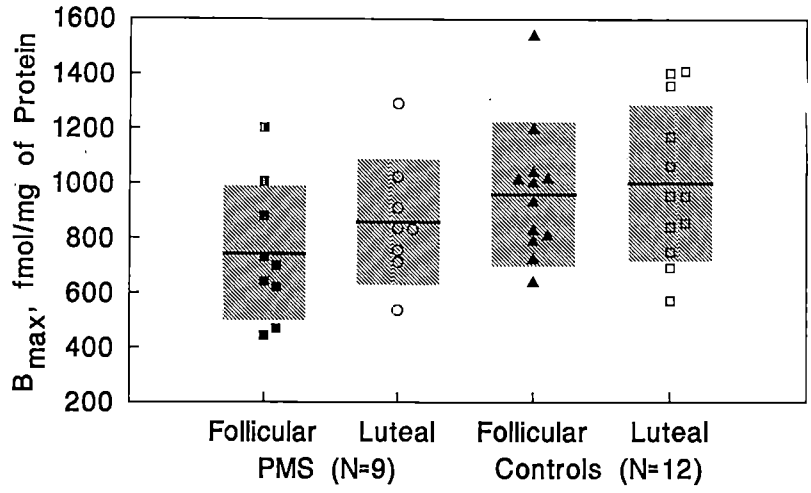


Fig. 1. [^{3}H]Imipramine binding sites in controls and women with premenstrual syndrome by menstrual cycle phase. *Bars,* means; *shaded areas,* ± 1 SEM. Only paired data points were used for statistical analysis.

cycle phase. Pearson's r was used to measure the correlation between luteal platelet binding site density and Beck Depression Inventory scores from baseline cycles. The $p < 0.05$ level of significance was used.

Results

Control subjects had, as expected, normal Beck Depression Inventory scores in the follicular (1.0 ± 1.2, mean ± SD) and luteal (2.4 ± 3.2, mean ± SD) phases of a baseline charting cycle. PMS subjects were normal in the follicular phase but were significantly depressed in the luteal phase; this was demonstrated by increased Beck Depression Inventory scores (2.1 ± 2.90 vs 17.1 ± 10.0, $p < 0.001$). Four of the nine PMS subjects had luteal phase Beck scores in the clinically depressed range (>16).

The number of [^{3}H]imipramine binding sites in samples obtained before and after the laboratory sessions did not differ significantly. This attests to the stability of platelet binding site characteristics over short time intervals, i.e., test-retest reliability. The mean value for each day was therefore used for subsequent analyses. For the analyses of variance, one subject was dropped from each group as a result of our inability to obtain samples in both menstrual cycle phases.

When follicular and luteal phase values within each subject group were combined, platelet [^{3}H]imipramine binding site density for PMS women was significantly lower than that of controls (800.2 ± 237.8 vs 981.4 ± 258.5 fmol/gm protein [$F(1,39) = 5.13$, $p = 0.03$]). Repeated measures analysis of variance revealed a time effect at the trend level ($F[1,17] = 2.57$, $p = 0.12$), but no time × group effect.

When platelet binding site number by cycle phase across subject groups was considered, (Fig. 1) women with PMS had lower maximum binding values than did controls in the follicular phase (744.8 ± 247.6 vs 963.0 ± 240.9 fmol/gm protein) by analysis of variance ($F[1,20] = 4.12$, $p = 0.057$) and Duncan's one-tailed t test ($p < 0.05$), but the two groups were not different in the luteal phase (862.4 ± 225.4 vs 1006.3 ± 284.2 fmol/gm protein; $F[1,18]$, $p = 0.27$, and $p > 0.05$, respectively). The increase in the number of [^{3}H]imipramine binding sites in platelets in PMS women from the follicular to the luteal phase was statistically significant (750.5 ± 264.1 vs 862.4 ± 225.4 fmol/gm protein, $p < 0.05$).

Luteal phase Beck Depression Inventory scores were not correlated with either follicular or luteal platelet [^{3}H]imipramine binding site density in PMS subjects.

Comment

This study documents lower numbers of platelet [^{3}H]imipramine binding sites in women with well-documented PMS who, in the follicular phase of the menstrual cycle, were clearly free of depression by stringent clinical and psychometric criteria. In results consistent with those of previous reports,[13, 14] asymptomatic women showed no influence of menstrual cycle phase on platelet [^{3}H]imipramine binding. Subjects in the present study with late luteal phase dysphoric disorder had lower values in the follicular as opposed to the luteal phase. Rojansky et al.[15] also noted differences in [^{3}H]imipramine binding in controls as opposed to women with "premenstrual changes," but they noted lower maximum binding values in early versus late luteal phase samples from symptomatic women. However, they observed a significant cycle phase effect on maximum binding in controls, a change that was not observed in this study or in previous reports.[13, 14] Their

subject population included some women with a lifetime diagnosis of depression, but this study excluded those women. The large degree of variability in the assay results and the limited number of times of sampling may further account for these apparent discrepancies.

The density of [3H]imipramine binding sites has been reported to be reduced in depressed patients in the majority of studies[8] and in scattered reports of a few possibly related conditions such as anorexia,[16] agoraphobia,[17] and obsessive-compulsive disorder.[16] The latter two disorders are among those overrepresented in an epidemiologic study of psychiatric disease and premenstrual symptoms (Duke Epidemiologic Catchment Area Study. Unpublished data). The number of platelet [3H]imipramine binding sites was about 18% lower in women with PMS than in controls; this was a smaller difference than the average 35% reduction seen in studies of depressed patients, which suggests that PMS may be a variant of depression modulated by the menstrual cycle.

The observation of a strong trend toward reduced binding sites to the serotonin transporter in women with PMS during their asymptomatic times is of interest. Platelets have a mean life-span of 21 days. Because [3H]imipramine binding sites are cell membrane characteristics of the anucleate platelet, they may be seen as evidence of synthetic events that occurred weeks earlier in the megakaryocyte. Low-follicular-phase density of the binding sites and the emotional state of the preceding luteal phase may both be the results of luteal phase biosynthetic events. Although previous studies have shown that platelet [3H]imipramine binding sites return to normal after the clinical resolution of depression, most researchers have measured this change 1 to 2 months after clinical improvement has occurred.[7, 18, 19] PMS may indeed provide a viable model for the study of affective state change that may resemble an acute depressive episode.

Sex hormones are well known to affect serotonergic systems. Pharmacologic doses of estrogen in rats[20] and physiologic replacement doses (10 mg of estradiol valerate monthly) in surgically menopausal women[21] increase the number of these binding sites approximately 20%. The time course of these changes is uncertain. In the latter study,[21] women given estrogen showed improved mood compared with that of their preoperative state, but controls failed to show any decline in mood while deprived of estrogen replacement. The precise relationship of estrogen levels to mood thus remains enigmatic with respect to both premenstrual and postmenopausal states.

Previous studies of serotonergic mechanisms in PMS have yielded contradictory findings. Whole blood serotonin levels[22, 23] and 5-hydroxytryptamine[24] uptake are reported to be lower in premenstrual women with premenstrual syndrome, and the maximum volume of platelet serotonin uptake is reportedly decreased premenstrually as well.[22] Malmgren et al.,[25] in a study limited by the absence of daily symptom ratings, compared women who had PMS with control subjects and found no effect of subject group or cycle phase on platelet serotonin uptake.

The lack of correlation of a state measure of depression (Beck Depression Inventory score) with the density of binding sites is consistent with reports of depressed patients in which psychometric measures of mood failed to correlate with this measure of presynaptic serotonergic function.[12] The high level of variance in binding site data in general makes it difficult to show such associations without studying large numbers of subjects and diminishes the potential clinical diagnostic value of this laboratory measure.

This study provides impetus for the further investigation of the dynamics of this presynaptic serotonergic binding site as a marker of affective state across the menstrual cycle and the relationship between premenstrual emotional changes and more continuous depressive disorders.

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The development of ultradian rhythms in the human fetus

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OBJECTIVE: Our objective was to determine the normal sequence of neurobehavioral development in the human fetus between 14 weeks' gestation and delivery.
STUDY DESIGN: The study was performed by longitudinal ultrasonographic observation of 45 low-risk singleton fetuses.
RESULTS: During the first half of the midtrimester there was a high rate of spontaneous movement that appeared randomly distributed. By the end of that trimester an increase in the duration of intervals of quiescence resulted in activity appearing cyclically distributed, with the duration of quiet cycles progressively increasing to term. Fetal mouthing and breathing were linked with cyclic behavior from the time of their emergence. Fetal heart rate pattern A could be recognized from around 32 weeks, due to a reduction in baseline variability in quiet cycles after 30 weeks, whereas pattern B could be recognized several weeks earlier. From the time cyclic behavior and heart rate patterns could be recognized, intervals of coincidence of the fetal behavioral state variables increased progressively with advancing gestation. (Am J Obstet Gynecol 1992;167:172-7.)

Key words: Fetal behavior, ultradian rhythms

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The first half of pregnancy is characterized by rapid emergence of a wide variety of fetal movements of relatively high frequency but little neurologic organization.[1, 2] During the second half of pregnancy these movements become organized. Diurnal rhythms are not demonstrable at 13 weeks but have been reported

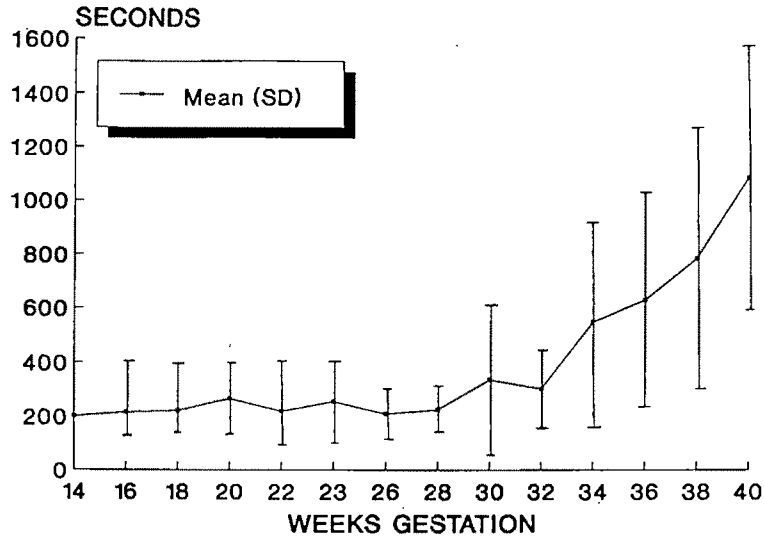


Fig. 1. Duration (mean and SD) of longest interval of complete quiescence during 100-minute longitudinal recordings between 14 weeks' gestation and term.

at 20 to 22 weeks,[3] 24 to 28 weeks,[4] and during the last 10 weeks of pregnancy.[5] All of these studies found peaks of fetal activity occurring in the late evening, in keeping with the commonly reported pattern of maternally perceived movements. These diurnal fluctuations are generally thought to be under maternal control, being related to diurnal fluctuations in maternal cortisol production[6] and inhibited by suppression of these fluctuations with exogenous steroids.[7]

More short-term, or ultradian, fluctuations in fetal activity also occur. In late pregnancy these are manifested as different behavioral states[8] that are very comparable to behavioral states of the newborn infant.[9] The stability, close linkage, and simultaneous change of the conditions of the different behavioral state variables is present in healthy fetuses from around 36 weeks' gestation. Before this, linkage of the variables is less consistent, but fluctuations in activity or rest-activity cycles are evident throughout the last trimester and sometimes even earlier.

The aims of the current study were to describe the development of these ultradian rhythms in the human fetus.

Patients and methods

A total of 21 primigravid and 24 multigravid women with no risk factors for fetal growth retardation, preterm delivery, gestational diabetes, or hypertension was recruited for longitudinal study of multiple fetal variables. Recordings began between 14 and 18 weeks' gestation and were repeated monthly until delivery.

Fetal diaphragmatic movements, limb, body, mouthing, and eye movements, were detected with real-time ultrasonography and recorded polygraphically at a paper speed of 3 cm/min (Hewlett Packard 7754A). The fetal heart rate (FHR) was also recorded ultrasonically with a standard FHR monitor (Hewlett Packard 8040A) with an output to the polygraph to allow synchronizing of all variables. During most of the second trimester recordings were made with a single scanner (Siemens Sonoline SL1 with 3.5 MHz transducer). However, in the last trimester two scanners were used, one giving a parasagittal view of the fetal face (Siemens Sonoline SL1 with 3.5 MHz transducer) and the other a transverse view of the fetal abdomen (Toshiba SAL 20A with 2.4 MHZ transducer). Fetal eye, somatic, and mouthing movements; breathing; and hiccups were recorded on separate channels of the polygraph with multiple hand-held push buttons.

Recordings were made with the patient in a comfortable, semirecumbant position for which we found a Parker Knoll chair most suitable. Recordings began between 9 AM and 6 PM and continued for 90 to 100 minutes. Within individuals, recordings were repeated at the same time of day and at the same interval after meals, and the small minority of mothers who smoked did not do so during the recording or for 2 hours before the recording.

All participants were outpatients with no risk factors known to affect fetal behavior or breathing. The following definitions were used:

Rest-activity cycles: A relatively high base rate of fetal movements interrupted by periods of quiescence lasting ≥6 minutes but which may be interrupted by a single startle.

Behavioral states: Stable association of heart rate pattern

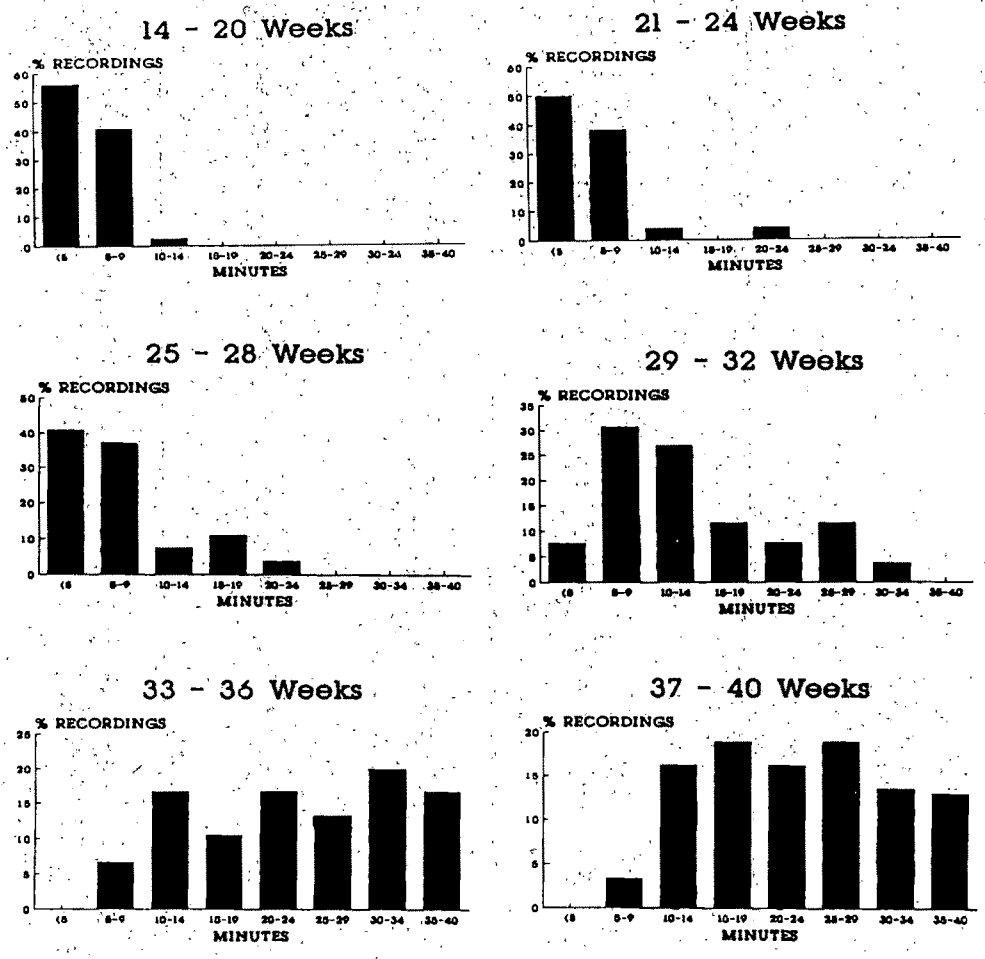


Fig. 2. Distribution of longest intervals of quiescence by gestational age grouping.

and condition of eye and somatic movements for intervals of ≥3 minutes with simultaneous transition (usually within 3 minutes) of all three variables at times of state transition.[8]

Fetal breathing: Contraction of the diaphragm resulting in downward and outward displacement of the abdominal contents and inward displacement of the thorax. All episodes of fetal breathing were counted provided they contained a minimum of four consecutive breaths sustained for ≥5 seconds. All episodes where the breath-to-breath interval was ≥6 seconds were excluded.[10] Isolated diaphragmatic movements were not counted.

Statistical analysis of the data was performed with analysis of variance and the paired *t* test.

Approval for the study was obtained from the hospital's research and ethical committee.

Results

The total number of recordings was 268 (mean 6.0, range 4 to 7 per fetus), and the total recording time was 402.4 hours.

Quiescence. Before 28 weeks the longest intervals of quiescence were around 200 seconds (mean 191 to 271 seconds), so that activity appeared randomly distributed with a high base rate of activity and very little quiescence. The longest interval of complete quiescence increased significantly ($p < 0.001$) with advancing gestational age after 28 weeks (Fig. 1).

The longest observed intervals of quiescence were allocated to 5-minute intervals between 0 to 5 and 35 to 40 minutes (only one quiescent interval lasted >40 minutes). Within six different gestational age groups the number of individuals with a particular interval of quiescence was plotted (Fig. 2). Before 24 weeks the majority of longest identifiable quiet intervals were <5 minutes, whereas after 32 weeks most quiet intervals were between 10 and 35 minutes. Although this clearly shows that fetal inactivity is gestional age dependent, evaluating only complete inactivity may not be an appropriate way to discriminate intervals of rest and activity. In the newborn, quiet (non–rapid eye movement) sleep may be interrupted by startles without a change of state occurring; we have therefore included

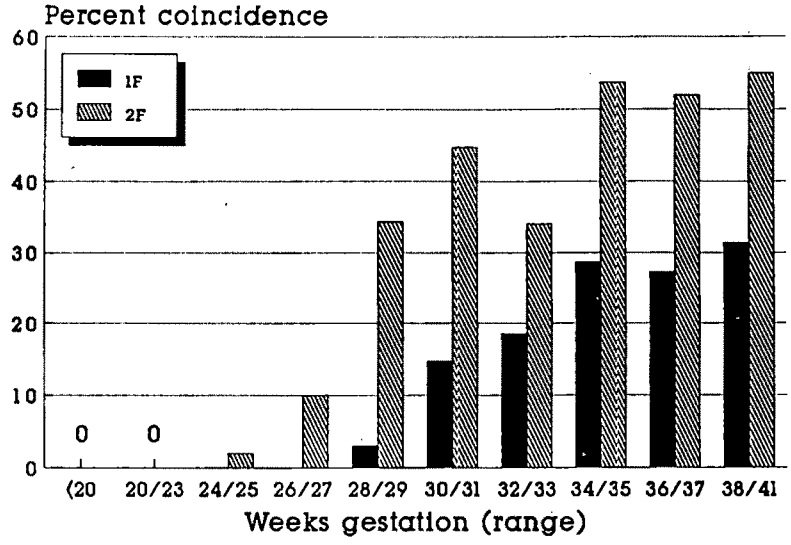


Fig. 3. Mean percentage coincidence 1F and 2F between 20 weeks' gestation and term.

quiet intervals interrupted by a single burst of movement in the fetus.

Rest-activity cycles. With the arbitrary definition of 6 minutes' total quiescence or interrupted by a single burst of movement for the minimum duration of a rest cycle, rest-activity cycles were rarely observed before 24 weeks, but by 29 weeks they were present in >80% of recordings.

Behavioral states. Fixed and recurring associations of body movements, eye movements, and the FHR pattern into well-defined fetal behavioral states were first observed in one fetus at 33 weeks and were present in >80% of recordings after 36 weeks. We have previously drawn attention to the fact that fetuses demonstrating state behavior may not consistently complete state transitions in a 3-minute interval.[11] We have therefore included recordings in this group, provided there was coincidence of the state variables for >90% of the recording and completion of at least one transition within 3 minutes.

Before the development of behavioral states there were intervals during which the behavioral variables would coincide in the conditions required for state behavior, although the heart rate patterns were never as distinct as they were after the development of states. Brief intervals of coincidence 2F and 1F were first observed in one fetus at 25 and 29 weeks, respectively. The percentage coincidence 1F and 2F is plotted in Fig. 3.

Eye movements. For determination of presence or absence of eye movements those intervals during which the eye was clearly visible were divided into 30-second epochs. Eye movements were considered intermittent if one to four occurred per epoch and continuous if there were more than five per epoch.

At all gestations eye movements tended to be absent during prolonged intervals of inactivity. During the first half of the middle trimester eye movements were often absent during episodes of activity and when present were intermittent. Towards the last trimester eye movements became more consistently associated with episodes of movement, and throughout the last trimester the percentage of active epochs with continuous eye movements progressively increased.

FHR patterns. FHR patterns were considered present once the baseline variability in quiet cycles was visibly narrow compared with that in active cycles (in practice a mean difference in variability ≥3 beats/min). By definition it follows that they could not be recognized in recordings without cycles; however, they were usually present several weeks before behavioral states.

State transitions. Consideration of the order of change of variables at the time of state change revealed that, at the gestational age at which states are present, FHR accelerations and fetal movements were so closely linked that the baseline variability was obscured by the presence of an acceleration at the onset of fetal movement. Thus it was impossible to determine if the FHR pattern and fetal movements changed simultaneously.

Fetal breathing. Throughout the gestational range studied fetal breathing movements were significantly more common in the active cycles ($p < 0.001$); however, within individuals this finding was not consistent with six recordings containing more breathing in quiescence than during the active cycles.

Fetal mouthing. Mouth opening, yawning, and tongue protrusion were observed throughout the gestational range studied. When rest and activity cycles were apparent in recordings these types of mouthing movements were almost confined to active cycles of

behavior. A very different type of mouthing, characterized by recurrent bursts of rhythmic movements of the tongue and mandible at a frequency of 1 to 3 Hz was observed once these were established. Rhythmic mouthing was observed during 67% of quiet cycles and 75% of intervals of state 1F, when the mouth was visible. Late in the second and early in the third trimester the duration of each burst was short, typically 1 to 2 seconds three or four times per minute. However, from around 34 weeks the burst duration was more variable, 2 to 15 seconds two to four times per minute.

Fetal outcome. All participants were delivered at term. All 1-minute Apgar scores were >5 and 5-minute scores were >7. The mean birth weight was 3532 gm (SD 435 gm, range 2700 to 4650 gm). One infant was admitted to the neonatal unit for investigation of pyrexia, which resolved without treatment, and in one other infant sudden, unexplained death occurred at 8 weeks. Careful retrospective examination of the recordings from this fetus did not reveal any differences from the other fetuses studied. With this one exception all infants are currently between 2 and 3 years of age and are developing normally.

Comment

We have previously shown that if biophysical evaluation of fetal well-being happens to coincide with state 1F, an abnormal or suboptimal result is obtained in a high percentage of normal fetuses.[12] The results of these observations reveal the converse at earlier gestations. Rest cycles, if present at all, are of short duration, and consequently observation of as few as three movements in a 30 minute interval (as recommended in the biophysical profile[13]) can hardly be considered a reassuring sign of well-being.

In the middle trimester there is an increase in baseline variability in both active and quiet cycles, whereas in the last trimester there is a significant reduction in baseline variability in quiet or 1F cycles with advancing gestational age beyond 30 weeks.[14] It is this which accounts for the appearance of the different heart rate patterns (A and B) previously described by Nijhuis et al.[8] It also accounts for the failure to identify intervals of 1F before 30 weeks, whereas brief intervals of 2F could often be identified several weeks earlier.

Although there is evidence that diurnal fluctuations are under maternal control,[6, 7] as yet it is unknown whether ultradian rhythms are under anything other than fetal control. We have previously reported absence of cyclic behavior in an abnormal fetus at 36 weeks, in whom FHR monitoring and the biophysical profile satisfied normal criteria.[15] Rhythmic mouthing movements have previously been reported as a concomitant of state 1F,[16, 17] and as with the state variables its presence during appropriate (quiet) epochs increased with advancing gestation. We have previously suggested that, because this is the fetal activity most frequently observed during quiet behavior, it would be useful to investigate its potential to discriminate between the quiet healthy fetus and one that is sick.[18]

Although clear trends were observed in human fetal behavioral maturation, the most consistent difficulty encountered was the inability to assign normal criteria for behavior for any particular gestational age. For example, although as a rule fetuses did not exhibit cyclic behavior before 24 weeks, six recordings did fulfill the criteria for a rest cycle, and two of these recordings were earlier than 17 weeks. Observations on a fetus not included in this study, but in whom fetal movement was absent during a routine scan lasting 20 minutes at 18 weeks, revealed only two sluggish movements during 45 minutes of recording at 19 weeks. The mother was well, did not smoke, drink, or take any drugs, and serial recordings throughout the remainder of pregnancy were completely normal, as was the baby at birth and at the age of 8 months.

Indeed, one of the most striking features of normal fetal behavior is its diversity. In terms of quantitative evaluation almost anything is acceptable, and gross deviation is necessary before it is recognizably abnormal. The lack of organization of behavior in early pregnancy makes identification of abnormality particularly difficult, although excessively forceful and monotonous movements have been noted in anencephalic fetuses.[19] In later pregnancy qualitative evaluation of coincidence of behavioral variables has revealed differences between groups of normal fetuses and fetuses subject to high maternal alcohol intake,[20] hydrocephalic fetuses,[21] fetuses of diabetic mothers.[22] and growth-retarded fetuses.[23] However, the wide range of coincidence found in normal fetuses at any particular age may render such observations valueless as a screening test for anything other than very severe neurologic impairment.

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Effect of calcitonin gene-related peptide on the uterine vasculature of the nonpregnant ewe

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OBJECTIVES: The current study was designed to evaluate the uterine vascular effects of calcitonin gene-related peptide on the uterine vasculature of nonpregnant sheep and compare them with the effects of prostacyclin.
STUDY DESIGN: Five nonpregnant oophorectomized ewes were instrumented with uterine and pulmonary artery flow probes and catheters. Dose-response curves were constructed according to increasing doses of calcitonin gene-related peptide (0.01, 0.03, 0.1, 0.3, 1, and 3, μg/min) and prostacyclin (0.03, 0.1, 0.3, 1, 3, and 10 μg/min) via 10-minute uterine artery infusions.
RESULTS: Both calcitonin gene-related peptide and prostacyclin produced a significant increase in uterine blood flow and a decrease in uterine vascular resistance. Calcitonin gene-related peptide was found to be approximately 17 times more potent than prostacyclin as a vasodilator. Local uterine artery infusions of calcitonin gene-related peptide led to significant increases in heart rate but did not alter blood pressure, cardiac output, or total peripheral resistance at the doses tested. In contrast, at doses of prostacyclin that produced similar uterine vasodilatation, prostacyclin led to significant decreases in systemic arterial blood pressure and total peripheral resistance and increases in heart rate and cardiac output.
CONCLUSION: These data strongly suggest that calcitonin gene-related peptide, an endogenously occurring vasoactive peptide, could play an important role in regulating uterine and systemic hemodynamics. (Am J Obstet Gynecol 1992;167:178-84.)

Key words: Sheep, prostacyclin, calcitonin gene-related peptide, uterine blood flow, vasodilatation

Calcitonin gene-related peptide is a newly discovered 37-amino-acid peptide translated from the calcitonin gene.[1] Immunohistochemical studies showed that calcitonin gene-related peptide–containing fibers are found in various perivascular nerves. The major role of circulating calcitonin gene-related peptide released from these peripheral nerves is thought to be control of organ blood flow. There is mounting evidence implicating calcitonin gene-related peptide as a potent vasodilator in the cardiovascular system.[2-6] Calcitonin gene-related peptide–containing nerve fibers have been localized in the nonpregnant guinea pig uterus and uterine artery.[7, 8] In addition, circulating levels of calcitonin gene-related peptide have been reported to be significantly elevated in pregnancy with the highest concentration occurring close to term.[9] However, our knowledge of the vasoactivity of calcitonin gene-related peptide in the uterine vasculature is currently limited to in vitro experiments.[10, 11] Thus these experiments were designed to determine the effects of calcitonin gene-related peptide on the uterine vasculature using a chronically catheterized nonpregnant sheep preparation.

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Material and methods

Surgical procedures. Five parous, nonpregnant sheep of mixed breed weighing 47 to 55 kg were used in this investigation. All animals underwent two operations, which are described below.

Thoracotomy. Before operation the sheep were fasted for 48 hours and water was withheld for 24 hours. Animals were initially anesthesized with pentobarbital (12 mg/kg), intubated, and placed on a ventilator with 3% to 4% isoflurane and oxygen to maintain anesthesia. The pulmonary artery was exposed via a left lateral thoracotomy and fitted with a 24 mm Doppler ultrasonographic flow probe (Transonic Systems, Inc., Ithaca, N.Y.) for determination of cardiac output. The thorax was closed in layers and drained by catheter to continuous vacuum suction for 3 days. The probe

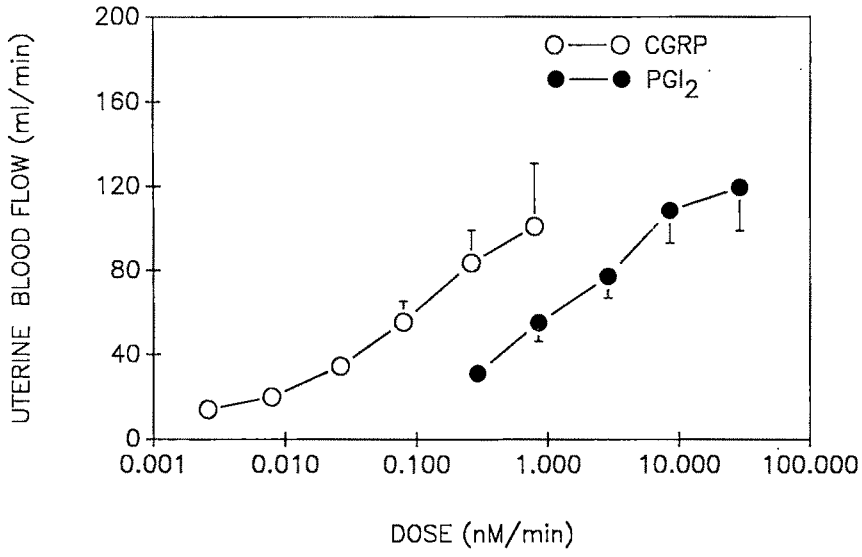


Fig. 1. Dose of calcitonin gene-related peptide *(open circles)* and prostacyclin *(solid circles)* versus increases in uterine blood flow (milliliters per minute) in nonpregnant ovine preparation ($n = 5$ for both compounds at all doses except for calcitonin gene-related peptide at highest dose where $n = 3$). Values are means ± SEM. Calcitonin gene-related peptide and prostacyclin induced significant increases in uterine blood flow ($p = 0.0001$).

cable was tunneled subcutaneously to exit the left flank and stored in a canvas pouch. After the operation the ewes were housed in individual portable cages and provided with commercial feed and water ad libitum.

Abdominal preparation. Subsequent to a 5- to 7-day recovery period from thoracotomy, sheep were fasted and anesthetized as described previously. Through a left groin incision, polyvinyl catheters were inserted into the ewe's femoral artery and vein to the level of the distal aorta and vena cava respectively. These catheters were used to monitor arterial blood pressure or for systemic administration of drugs. The uterus was exposed by a midline incision, and electromagnetic flow probes were placed on both uterine arteries. To allow direct intraarterial administration of compounds, the lateral branch of each uterine artery was catheterized distal to the flow probe. All animals underwent ovariectomy to prevent cyclic fluctuation. The incision was closed, and catheters and cables were passed through a subcutaneous tunnel, exteriorized through a small incision on the ewe's flank, and stored in a canvas pouch attached to the ewe's side. All catheters were flushed every other day with heparin (1000 U/ml) to maintain patency. Each ewe received an intravenous injection of 17β-estradiol (1 μg/kg) each day to prevent uterine atrophy.

Experimental procedure. Experiments were not performed before the fifth postoperative day after the second operation to assure full recovery from surgical stress. After a 30-minute baseline recording period of arterial pressure, heart rate, cardiac output, and uterine blood flow, calcitonin gene-related peptide or prostacyclin was administered as a continuous 10-minute infusion through either the left or right uterine artery catheter. Compounds were infused at the rate of 0.1 or 0.3 ml/min. Cumulative dose-response curves were obtained for calcitonin gene-related peptide (0.01 to 3 μg/min) and prostacyclin (0.03 to 10 μg/min) on separate days. At the end of each experiment animals were given 1 μg/kg of 17β-estradiol via the femoral vein to evoke a maximum uterine blood flow response, which allowed us to normalize our data and evaluate the uterine potency of calcitonin gene-related peptide and prostacyclin.

Physiologic measurements. Uterine blood flow was measured by a square-wave electromagnetic flowmeter (RF 2100, Dienco, Los Angeles) in all five sheep, and cardiac output was measured in three of the five sheep with a Doppler ultrasonographic flowmeter (Transonic T 101, Transonic Instruments, Ithaca, N.Y.). Arterial blood pressures were monitored by Micron MP 15 pressure transducers (Micro Instruments, Simi Valley, Calif.), and heart rate was determined by cardiotachometer couplers (Sensor Medics, Anaheim, Calif.) triggered by arterial pressure pulses. All parameters were continuously recorded with Sensor Medics R612 physiologic recorders. Total peripheral and uterine vascular resistance was computed from mean arterial pressure and cardiac output or uterine blood flow, respectively, with no correction being made for central venous pressure.

Drug preparations. Calcitonin gene-related peptide

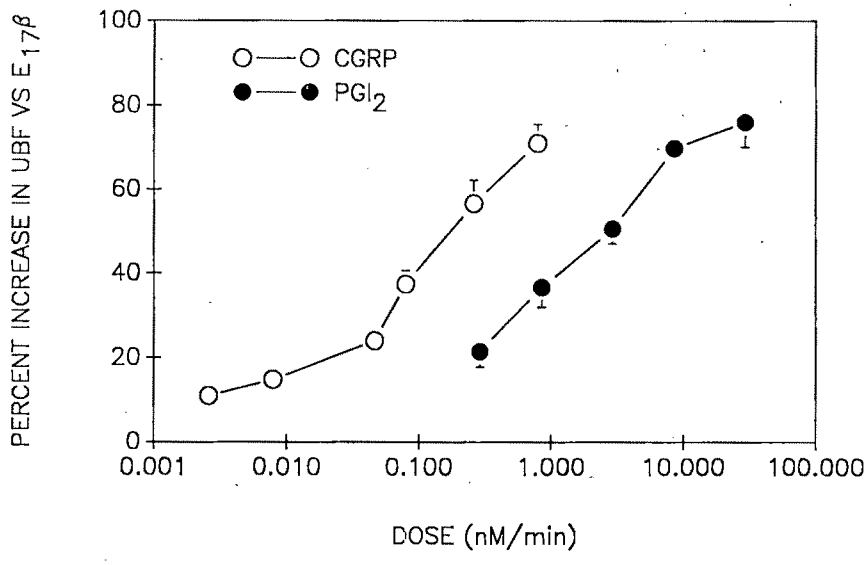


Fig. 2. Dose of calcitonin gene-related peptide *(open circles)* and prostacyclin *(solid circles)* versus percent of uterine blood flow response to 17β-estradiol in nonpregnant ovine preparation ($n = 5$ for both compounds at all doses except for calcitonin gene-related peptide at highest dose where $n = 3$). Values are means of individual percent changes ± SEM. At highest doses of calcitonin gene-related peptide and prostacyclin tested uterine blood flow was increased to 71% ± 5% and 76% ± 6% of maximum uterine response to estrogen.

(Peptides International, Louisville, Ky.) was prepared as a stock solution of 0.1 mg/ml in distilled water and stored at −20° C until use, at which time it was serially diluted with normal saline solution to the desired concentrations and kept on ice until used. Prostacyclin sodium salt (The Upjohn Company, Kalamazoo, Mich.) was prepared fresh immediately before use by dissolving it in 0.01 mol/L Tris buffer, pH 9.4, to prevent degradation.

Data analysis. The uterine vasodilator effects of calcitonin gene-related peptide and prostacyclin were expressed as both the absolute uterine blood flow changes (milliliters per minute) and as a percent of the maximum dilatation produced by 17β-estradiol and reported as mean ± SEM. In five sheep arterial pressure and heart rates were recorded, and in three of the five sheep cardiac output was recorded and total peripheral resistance was computed. All the parameters were measured during the last 2 minutes of each 10-minute infusion. Data were analyzed by one-factor analysis of variance, and the Newman-Keuls procedure was used to determine which mean was significantly different from baseline ($p < 0.05$).

Results

Effect of local intraarterial infusion of calcitonin gene-related peptide on uterine blood flow. Local intraarterial infusion of calcitonin gene-related peptide and prostacyclin produced significant dose-related increases in uterine blood flow in the nonpregnant ewes as illustrated in Fig. 1. Uterine blood flow increased from a baseline of 9 ± 1 ml/min to a maximum of 101 ± 30 ml/min during the infusion of 3 μg/min of calcitonin gene-related peptide. Responses reached a steady state by the end of each 10-minute infusion. Prostacyclin increased uterine blood flow from a baseline of 13 ± 1 ml/min to a maximum of 120 ± 21 ml/min at the 10 μg/min dose. On a nanomoles per liter per minute basis, calcitonin gene-related peptide was about 17 times as potent as prostacyclin in dilating the uterine circulation (Figs. 1 and 2). At the doses tested, calcitonin gene-related peptide (0.79 nmol/L/min = 3 μg/min) and prostacyclin (29 nmol/L/min = 10 μg/min) were able to increase uterine blood flow to 71% ± 5% and 76% ± 6% of the maximal estrogen response, respectively (Fig. 2). The vehicles, saline solution for calcitonin gene-related peptide or 0.01 mol/L Tris buffer for prostacyclin, did not significantly alter uterine blood flow at the infusion rates used. In animals receiving calcitonin gene-related peptide the calculated uterine vascular resistance decreased significantly from a baseline of 8.72 ± 1.18 to 0.84 ± 1.89 mm Hg/ml/min at the highest dose, whereas in animals receiving prostacyclin the uterine vascular resistance decreased from 6.23 ± 1.17 to 0.56 ± 0.10 mm Hg/ml/min at the highest dose infused (Fig. 3). The amount of time required for the highest dose of each vasodilator to decrease the half of

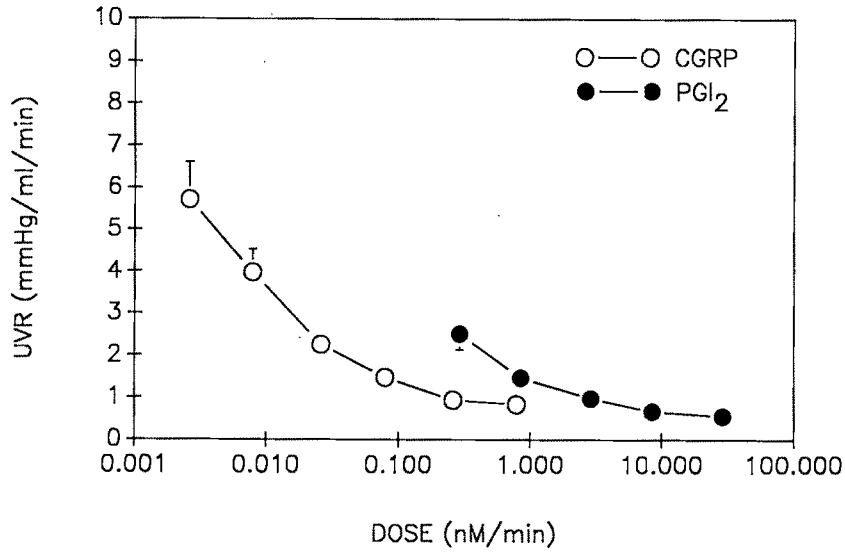


Fig. 3. Dose of calcitonin gene-related peptide *(open circles)* and prostacyclin *(solid circles)* versus decreases in uterine vascular resistance *(UVR)* in nonpregnant ovine preparation ($n = 5$ for both compounds at all doses, except for calcitonin gene-related peptide at highest dose where $n = 3$). Values are means ± SEM. Calculated uterine vascular resistance decreased significantly from a baseline of 8.72 ± 1.18 to 0.84 ± 0.18 mm Hg/ml/min at highest dose of calcitonin gene-related peptide infused and from 6.23 ± 1.17 to 0.56 ± 0.10 mm Hg/ml/min at highest dose of prostacyclin.

baseline after the completion of the 10-minute infusion was significantly longer for calcitonin gene-related peptide than prostacyclin (12 ± 1 vs 7 ± 2 minutes, $p = 0.02$).

Effect of intraarterially administered calcitonin gene-related peptide and prostacyclin on systemic hemodynamics. The local intraarterial infusion of calcitonin gene-related peptide led to significant increases in heart rate (94 ± 9 beats/min to 120 ± 6 beats/min) but did not significantly alter systemic arterial pressure, cardiac output, or calculated total peripheral resistance at the doses tested (Figs. 4 and 5). Because of the expense of purified calcitonin gene-related peptide higher doses were not investigated. Local infusion of prostacyclin led to a significant decrease in systemic arterial pressure (73 ± 4 to 59 ± 4 mm Hg) and total peripheral resistance (12.38 ± 0.65 to 8.39 ± 0.61 mm Hg/ml/min) with a significant increase in heart rate (92 ± 2 to 125 ± 5 beats/min) and cardiac output (5.8 ± 0.4 to 7.3 ± 0.4 L/min). Results are depicted graphically in Figs. 4 and 5.

Comment

The current work confirms and extends our knowledge about the effects of calcitonin gene-related peptide on the uterine vasculature. We have used a chronically instrumented unanesthetized nonpregnant sheep model in which we have administered calcitonin gene-related peptide directly into the uterine artery via an intraarterial infusion to determine its uterine vascular response. Our data showed that calcitonin gene-related peptide is a very potent vasodilator in the nonpregnant sheep uterus and is able to produce uterine vasodilatation, which is equivalent to that observed with 17β-estradiol. On a molar basis calcitonin gene-related peptide was about 17 times more potent than prostacyclin as a uterine vasodilator. On stopping the infusion of calcitonin gene-related peptide the duration of the vasodilatation produced was about 50% longer than that seen with prostacyclin. These data support the concept that calcitonin gene-related peptide is a potent uterine vasodilator and could possibly play an important role in the regulation of uterine blood flow in the nonpregnant and pregnant states.

Calcitonin gene-related peptide–containing fibers have been identified in nonpregnant guinea pig uterus and uterine artery by immunocytochemical examination.[7, 8] In addition, circulating plasma levels of calcitonin gene-related peptide have been reported to be significantly increased throughout pregnancy with the highest concentration close to term.[9] However, our knowledge of the physiologic significance of calcitonin gene-related peptide on the regulation of uterine blood flow in the nonpregnant or pregnant state is currently limited. Recently calcitonin gene-related peptide has been shown to be a potent vasodilator of in vitro preparations of preconstricted human and guinea pig uterine arteries.[10, 11] Nelson[10] showed that uterine arteries

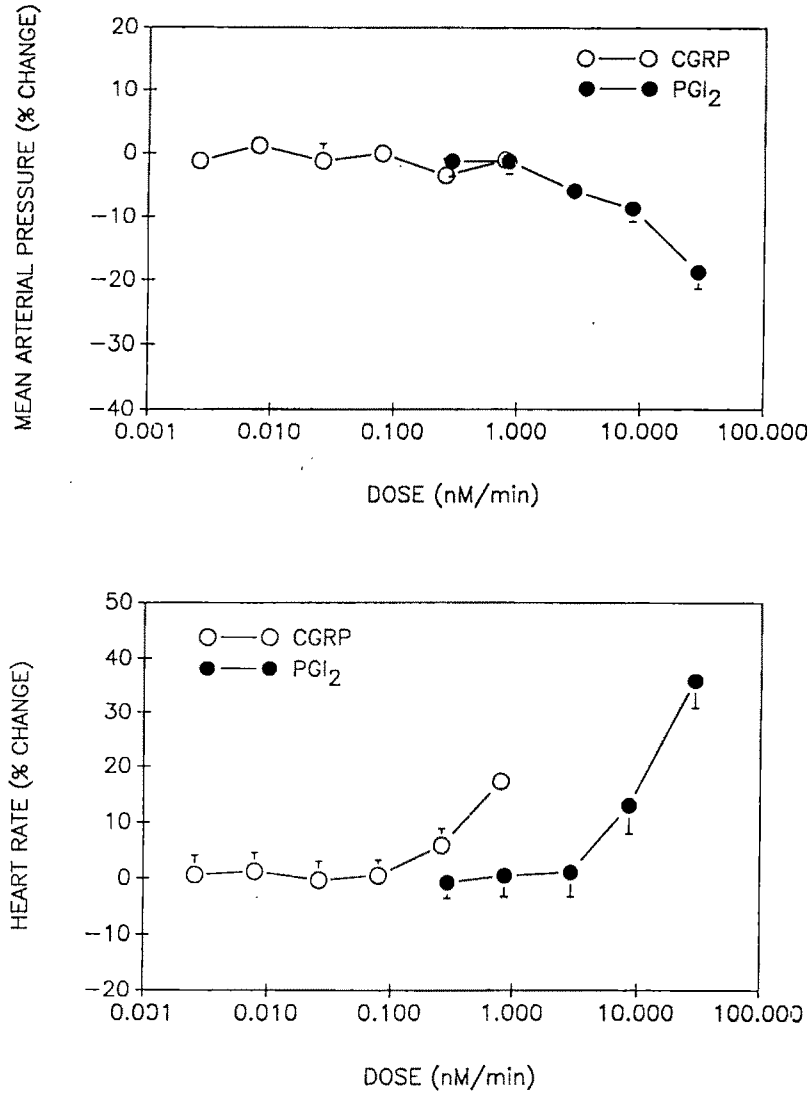


Fig. 4. Effect of local uterine artery infusion of calcitonin gene-related peptide and prostacyclin on mean arterial blood pressure *(upper panel)* and heart rate *(lower panel)*. Values are percent change from baseline ($n = 5$ for both compounds at all doses except for calcitonin gene-related peptide at highest dose where $n = 3$). Calcitonin gene-related peptide produced significant increases in heart rate ($p < 0.05$) at highest dose, whereas prostacyclin decreased blood pressure ($p = 0.0002$) and increased heart rate ($p = 0.0002$) in a dose-related fashion.

from pregnant women were more sensitive to the vasodilator effects of calcitonin gene-related peptide than were those from nonpregnant women, suggesting that calcitonin gene-related peptide might play an important role in regulating urine blood flow in pregnancy. Further investigations will be needed to assess the effects of calcitonin gene-related peptide on uterine blood flow in pregnancy.

Although calcitonin gene-related peptide is approximately 17 times more potent than prostacyclin as a vasodilator in the uterine circulation, systemic hemodynamic responses to the local uterine infusion suggest that the systemic vasculature may be less sensitive. While calcitonin gene-related peptide at the doses evaluated did not significantly change arterial blood pressure, prostacyclin significantly decreased systemic arterial pressure at the two highest doses. Both calcitonin gene-related peptide and prostacyclin significantly increased heart rate. This increased heart rate is most likely a compensatory response to peripheral vasodilatation produced by both compounds. Under the current protocol only prostacyclin produced significant increases in cardiac output and decreases in total peripheral resistance. The lack of systemic responses to calcitonin gene-related peptide at doses that are equally potent as vasodilators in the uterus can be interpreted

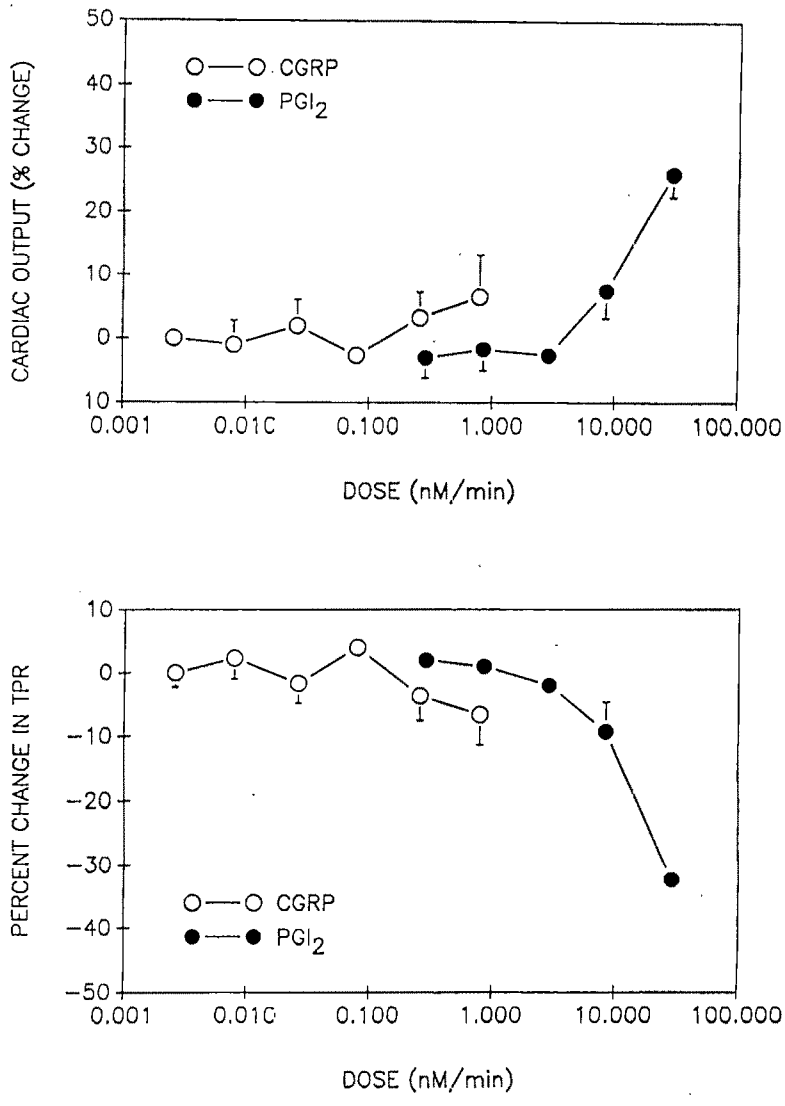


Fig. 5. Effect of uterine artery infusions of calcitonin gene-related peptide and prostacyclin on cardiac output *(upper panel)* and calculated total peripheral resistance *(lower panel)*. Values are percent change from baseline (n = 3 for both compounds at all doses). Prostacyclin produced a dose-related increase in cardiac output (p = 0.04), whereas calcitonin gene-related peptide had no significant effect on cardiac output at doses tested. Prostacyclin also produced a significant decrease in calculated total peripheral resistance (p = 0.003).

in two different ways. First, calcitonin gene-related peptide may be broken down or be metabolized before reaching the peripheral circulation, a possibility that does not seem likely because at least in humans it has a circulating half-life of 10 minutes[4] and in our study the uterine vascular response had a half-life of 12 minutes. A second possibility is that the systemic vasculature is less sensitive to calcitonin gene-related peptide than is the uterine vasculature. It appears (Figs. 4 and 5) that increasing doses of calcitonin gene-related peptide would have led to significant decreases in arterial blood pressure and total peripheral resistance, as well as increases in cardiac output. Thus, although the uterus appears to be more sensitive to calcitonin gene-related peptide than to prostacyclin, the systemic vasculature may not share this sensitivity. To determine this, higher doses of calcitonin gene-related peptide would have to be used.

In summary, in the current study we have investigated the vasoactive properties of calcitonin gene-related peptide on uterine vasculature. Calcitonin gene-related peptide appears to be a potent vasodilator agent in the ovine uterine vasculature. On a molar basis, it appears to be approximately 17 times more potent than prostacyclin, the most potent uterine vasodilator prostaglandin.[12] These properties suggest that this com-

pound could potentially play an important role in regulating uterine blood flow.

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An ultrastructural study of the developing urogenital tract in early human fetuses

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Detroit, Michigan, Philadelphia, Pennsylvania, San Francisco, California, Boston, Massachusetts, Newark, New Jersey, and Mie-Kan, Japan

OBJECTIVES: This study examines the gonoducts during the ambisexual stage of human fetal development to define their ultrastructural characteristics, including the origin and anatomic relationship of the mesonephric and paramesonephric ducts during gonoductal development.

STUDY DESIGN: The reproductive tracts from five fetuses ranging in gestational age from 35 to 45 days were processed for ultrastructural examination. The developing mesonephric ducts, paramesonephric ducts, and their surrounding mesenchyme were studied with a Phillips 300 transmission electron microscope.

RESULTS: The mesonephric ducts and paramesonephric ducts have distinctive cytoplasmic and cell surface ultrastructural characteristics, as well as different morphologic patterns of epithelial-mesenchymal interaction. Cephalad portions of mesonephric ducts and paramesonephric ducts are separated by mesenchyme, but more caudal aspects move progressively closer until they are juxtaposed but separate.

CONCLUSIONS: Early mesonephric ducts and paramesonephric ducts may be distinguished because of their distinctive ultrastructural features; epithelial-mesenchymal interaction may be important in their differentiation and maintenance; both gonoducts retain their morphologic identity throughout, supporting their separate origins. (Am J Obstet Gynecol 1992;167:185-93.)

Key words: Ultrastructure, fetus, urogenital, paramesonephric, müllerian, mesonephric, wolffian

The early reproductive tract in humans consists of two paired gonoducts. The mesonephric (wolffian) ducts develop first, arising around gestational day 24 from the paravertebral mesenchyme as a solid cord of cells. The ducts grow caudad, and around the 26th postovulatory day a lumen develops. By day 27 the mesonephric ducts have reached and opened into the cloaca, where they serve as the main excretory ducts of the embryo.

The paramesonephric (müllerian) ducts do not begin their development until postovulatory day 37, when they appear as funnel-shaped openings of the coelomic epithelium. The paramesonephric ducts appear to follow the mesonephric ducts like a "guidewire"; on day 48 the paramesonephric ducts are approximately halfway to the urogenital sinus and pass ventral to the mesonephric duct. The paramesonephric ducts continue to approach each medially and then grow to and contact the urogenital sinus at a ventral projection of its dorsal wall called the müllerian tubercle, which is situated between the openings of the mesonephric ducts. Around day 56, the paramesonephric ducts fuse to become the uterovaginal canal, the primordia destined to become the uterine corpus, cervix, and vagina. The upper unfused paramesonephric ducts become the fallopian tubes.

Although ultrastructural studies of the developing genital tract in experimental animals have been reported,[1, 2] to our knowledge no such studies of human material exist in the literature. This report, which is part of an ongoing study of the normal and abnormal development of the human genital tract,[3-5] presents our findings in five fetuses from approximately 35 to 45 postovulatory days of age. This period occurs during the ambisexual stage of gonadal development, during which the gonad is indifferent and not distinguishable morphologically as ovary or testis by traditional light

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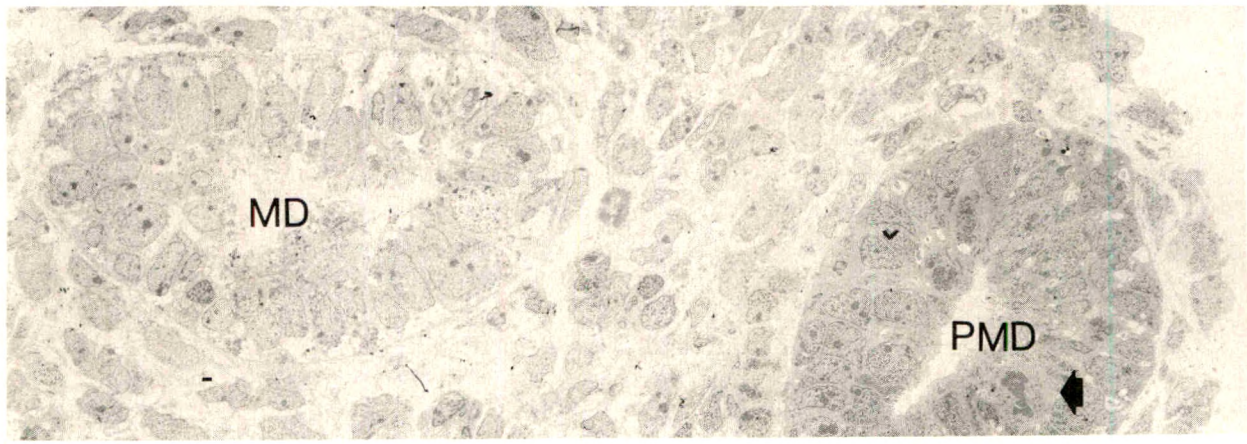


Fig. 1. Paramesonephric and mesonephric ducts. A mitotic figure *(arrowhead)* is present in paramesonephric duct *(PMD)*. Mesonephric duct *(MD)* has numerous subnuclear clear vacuoles corresponding to glycogen extracted during tissue processing. (×782.)

microscopy. In this communication, emphasis is placed on the ultrastructure of the developing mesonephric and paramesonephric ducts and their surrounding mesenchyme.

Material and methods

Human fetal material was obtained as described in detail elsewhere.[3] The ages of the fetal specimens, ascertained by heel-toe measurements,[6, 7] were 35 to 45 days of age (postovulatory). For such early specimens, determination of sex on morphologic grounds was impossible.

The reproductive tracts, which rarely exceeded 4 mm in length, were randomly placed in neutral buffered formalin for light microscopic analysis or were placed in Karnovsky's fixative for 2 hours at room temperature.

The specimens prepared for light microscopy were fixed in Bouin's solution, embedded in paraffin, sectioned at 6 μm, and stained with hematoxylin and eosin. In total, each genital tract yielded an average of 100 to 150 sections. The specimens prepared for transmission electron microscopy were divided after fixation into six to eight blocks. Sections of 1 μm stained with toluidine blue were prepared from each block, and where appropriate, ultrastructural analysis was performed using a Phillips 300 transmission electron microscope.

Results

Transmission electron microscopy of the gonoducts. The cells lining the early human paramesonephric duct are pseudostratified columnar in type, extending from the basal lamina to the lumen; cells are closely packed and nuclei are found at varying levels in the epithelium (Figs. 1 and 2). Apices of the cells exhibit numerous microvilli, many of which are elongate with rare terminal branching. Occasional cilia, although present, are poorly formed and lack the usual nine-plus-two internal arrangement. Apices of some cells bulge into the lumen in a manner reminiscent of the merocrine type of secretion observed in mature endometrial cells. Tight junctions are a prominent feature of the apical cell membranes. Desmosomes (macula adherentes) are present along the lateral borders of the cells, and are small and buttonlike. Nuclei of the paramesonephric duct epithelium show variability in size and shape and often contain one to three large nucleoli. The cytoplasm of the paramesonephric duct cells contains numerous polyribosomes and most cells contain from one to three Golgi complexes. Mitochondria are abundant. Lipid droplets are scattered throughout the cytoplasm and large lysosomes may be found in both supranuclear and subnuclear locations. A few cells contain subnuclear or supranuclear aggregates of glycogen. The basal aspect of the cells is relatively flat and cytoplasmic filaments may be present in a linear array adjacent and parallel to the basal lamina.

The stroma in the region of the developing paramesonephric duct is cellular and numerous mitotic figures are present. The stromal cells are large and epithelioid with abundant cytoplasm (Fig. 3). Nuclei are large with prominent nucleoli. The cell membrane is drawn out into fingerlike microvillus processes, which, over much of their length, lie in direct apposition to those of neighboring stromal cells. The cytoplasm contains long and short profiles of granular endoplasmic reticulum, polyribosomes, mitochondria, and scattered lipid droplets. The Golgi apparatus is well developed and is sometimes multiple. Small membrane-bound electron-lucent vesicles may be seen at the secretory end of the Golgi apparatus.

In contrast to the stroma around the mesonephric duct, the subepithelial stromal cells and their fine processes around the paramesonephric duct closely approach the base of the ductal epithelial cells and extend relatively short processes to make contact with the basal cell membranes. The basal lamina in these areas of close epithelial-stromal apposition appears incomplete or is absent (Fig. 3). The previously mentioned secretory vesicles appearing to arise from the Golgi apparatus may be numerous and sometimes fill the cytoplasm on the epithelial side of the stromal cell. Focally, the vesicles appear to merge with the stromal cell plasmalemma and others appear within the stroma as isolated membrane-bound vesicles. In paramesonephric duct epithelial cells, pinocytotic vesicles similar in size to those within underlying stromal cells appear at the basal plasmalemma, as well as in the basal portion of the cytoplasm. Some of the stromal cells contain cytoplasmic filaments, and poorly formed junctionlike attachments rarely are seen between stromal cells.

Mesonephric duct cells are tall, columnar and generally show less pseudostratification as compared with their paramesonephric counterparts (Fig. 4). The luminal border has short, stubby microvilli and the cilia are rudimentary, as in the paramesonephric ducts. The lumen focally contains degenerated and necrotic cell debris.

The most prominent feature of mesonephric duct cytoplasm is the presence of glycogen lakes, usually localized in a subnuclear position, although similar aggregations may be supranuclear (Fig. 4). Lipid droplets are less numerous than in the paramesonephric ducts. The cytoplasm of the epithelial cells contains prominent Golgi complexes, which are often concentrated in the apical portion. Polyribosomes are abundant and scattered diffusely throughout the cytoplasm.

When compared to the paramesonephric duct, the nuclear membrane of the mesonephric duct cells shows less irregularity and nucleoli are prominent. The basal aspect of the cell is generally flat. A delicate basal lamina underlies the base.

Large epithelioid stromal cells are present in the periductal stroma. Nuclei appear large, with prominent nucleoli. The cytoplasm contains numerous Golgi complexes, which, as in the stroma about the paramesonephric ducts, are more often polarized to the epithelial side of the cell. Polyribosomes are numerous and profiles of dilated rough endoplasmic reticulum contain a granular precipitate. Inclusions suggestive of primary lysosomes are found in scattered stromal cells, some of which show nuclear pyknosis and cytoplasmic degeneration.

Most of the stromal cells associated with the mesonephric ducts have long attenuated cell processes that

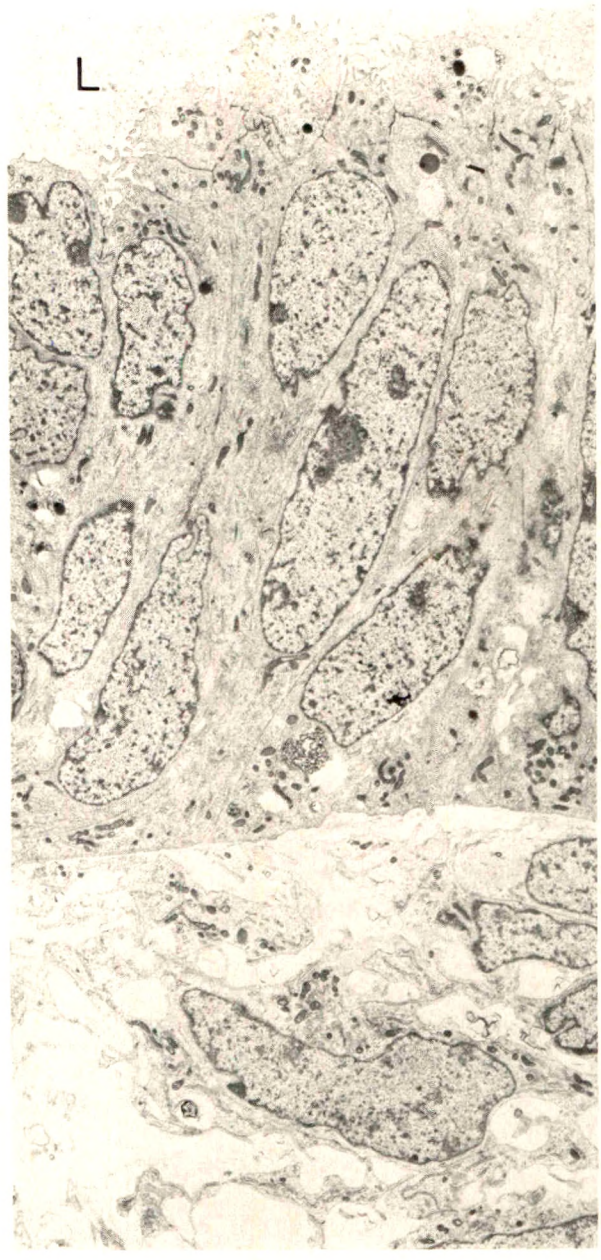


Fig. 2. Paramesonephric duct. Cells show pseudostratification; nuclei are large and contain prominent nucleoli. Microvilli extend from cell apices into lumen *(L)*. (×2548.)

interdigitate with those of neighboring cells, imparting the appearance of a network of subepithelial mesenchymal cells connected by delicate cell processes (Fig. 5). Often the cell body, which includes the nucleus, does not appear in a subepithelial zone of varying width. Consequently, this zone appears pale on light microscopy since it contains few nuclei and is composed primarily of delicate cell processes (Figs. 6 and 7).

Those stromal cells near the basal lamina of the mesonephric ducts have elongate fingerlike processes that

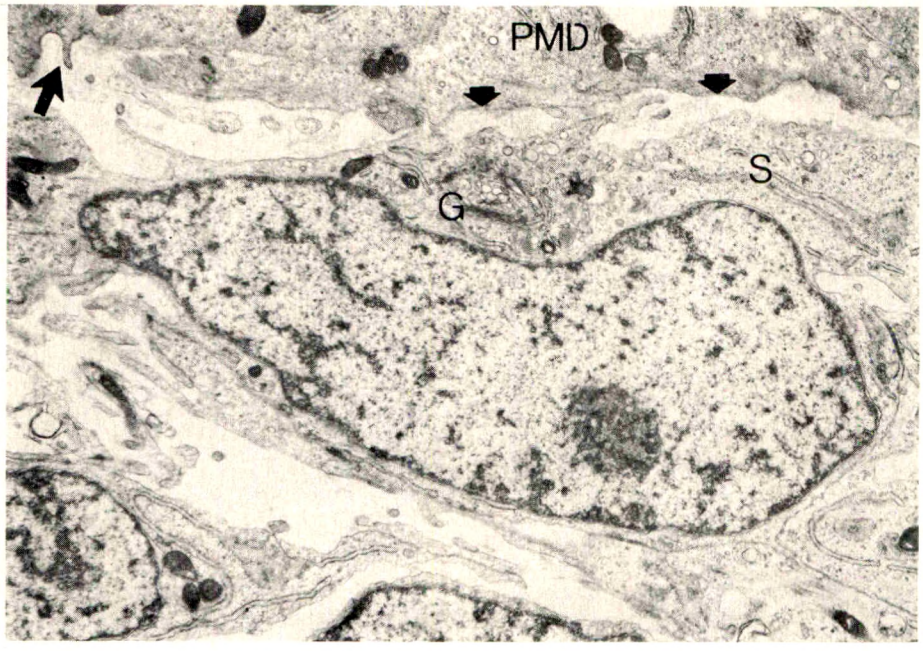


Fig. 3. Paramesonephric duct; epithelial-stromal junction. Note absence of basal lamina where epithelial stromal cell *(S)* closely approaches basal plasmalemma *(arrowheads)* of paramesonephric duct *(PMD)*. Delicate basal lamina *(large arrow)* is present at periphery. Stromal cell contains a large Golgi complex *(G)*. (× 10,200.)



Fig. 4. Mesonephric duct. Cells are columnar and appear less pseudostratified than paramesonephric duct. Lumen *(L)* contains granular precipitate. Subnuclear glycogen vacuoles *(V)* are prominent. Stromal cell processes closely abut basal lamina. (×3600.)

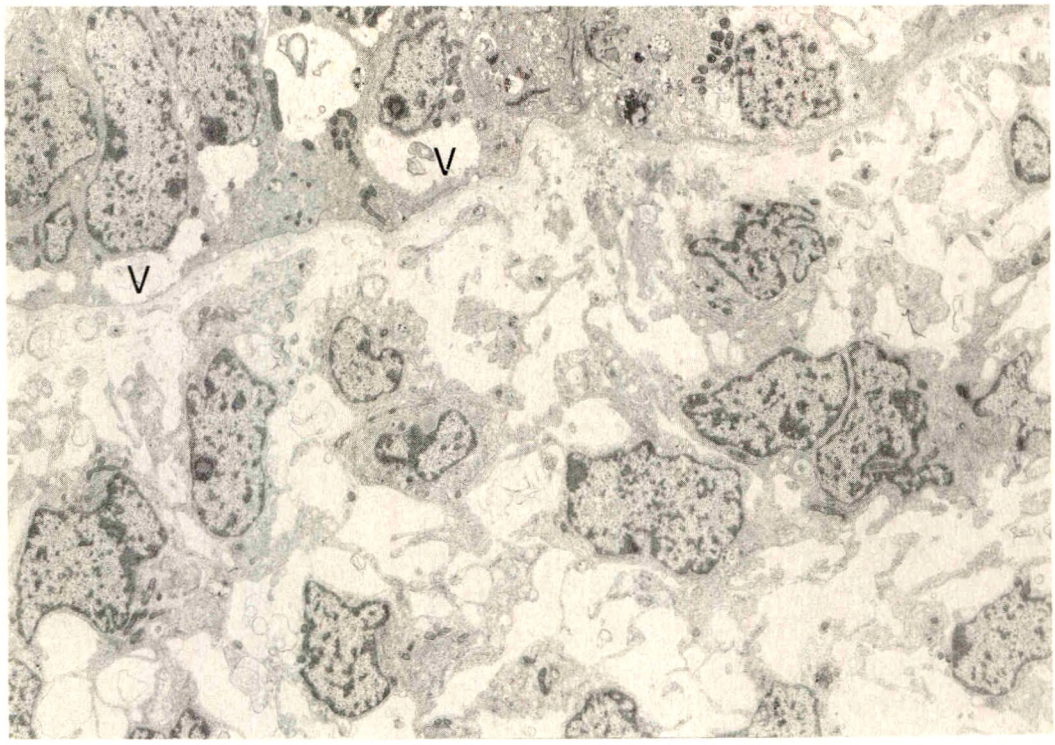


Fig. 5. Mesonephric duct. Stromal cells underlie mesonephric duct epithelium with subnuclear vacuoles *(V)*. Epithelioid stromal cells exhibit elongated cytoplasmic processes that interdigitate with neighboring cells. Nuclei are large, irregularly shaped, and contain prominent nucleoli. (×3913.)

extend to and make contact with the basal lamina (Fig. 6). In spite of the delicate nature of the cytoplasmic processes, they contact the basal lamina in such numbers that its surface is virtually covered, imparting a fine structural appearance reminiscent of podocytes in the renal glomerulus. This situation contrasts with the paramesonephric ducts, in which subepithelial stromal cells lie immediately subjacent to the basal lamina, accompanied by its focal dissolution. The basal lamina of the mesonephric duct appears continuous. There are, however, pinocytotic vesicles like those observed in the paramesonephric ducts present along the basal plasmalemma of the mesonephric duct; likewise, membrane-bound vesicles similar to those in stromal cells in the immediate vicinity of the paramesonephric ducts are observed in the stromal cells of the mesonephric duct.

In its most caudal portion, which represents the advancing edge, the paramesonephric duct is in direct apposition to the mesonephric duct (Fig. 8). In addition to their anatomic location, the gonoducts in this region are easily differentiated by the presence of abundant intracytoplasmic glycogen in the mesonephric ducts and its near absence in the paramesonephric ducts. In the advancing paramesonephric duct, cell membranes are tightly apposed, with complex interdigitations and sparse, poorly formed intercellular attachments. Numerous mitotic figures are present. Abundant microtubules are present throughout the cytoplasm.

At the point where the two gonoducts are immediately adjacent, mesonephric duct and paramesonephric duct cells are in intimate contact; cell membranes are directly apposed and no basal lamina is present between them (Fig. 9); basal lamina is present, however, in patches along the coelomic aspect of the advancing edge of the paramesonephric duct. Cytoplasmic filaments parallel to the long axis of the duct are present near the cell membrane in apposing ductal cells. Although focal thickening of the cell membrane is observed, the few intercellular attachments that are present lack the structure of a complete desmosome.

Comment

No differences in the light and ultrastructural features of the gonoducts could be discerned between sexes in genital tracts obtained during the ambisexual stage of development; during this time period, the developing testis has not yet produced müllerian-inhibiting substance, which induces regression of the paramesonephric duct.

The finding of comparable morphologic features between the early paramesonephric and mesonephric ducts of both sexes is similar to that observed in mice of 12 days' gestation,[1] in which the critical period of

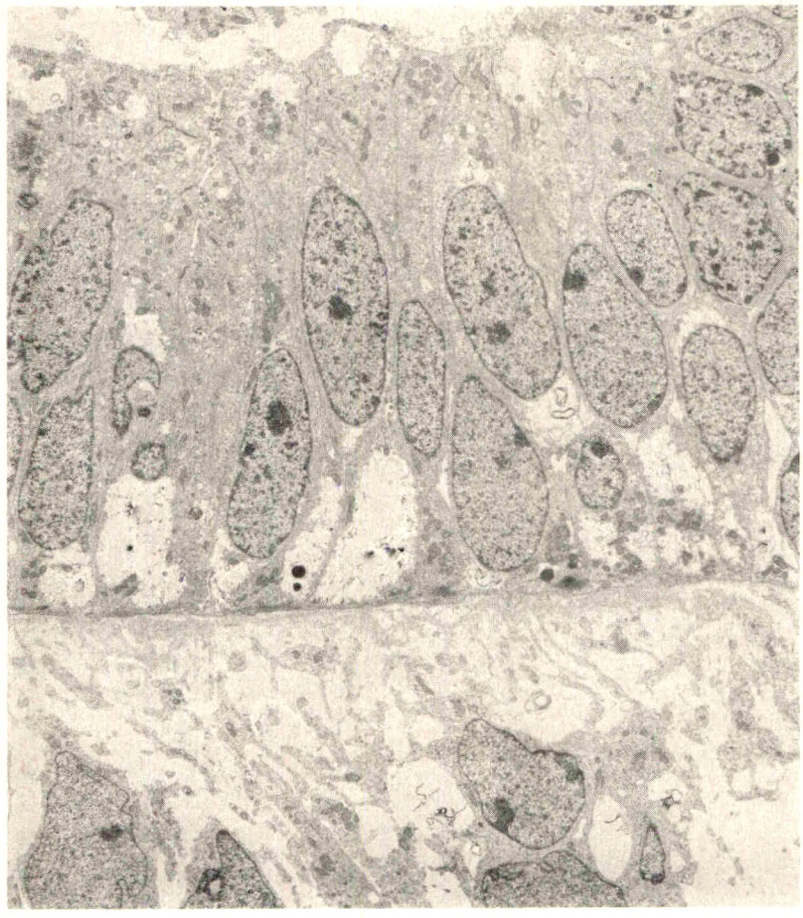


Fig. 6. Mesonephric duct. Subepithelial zone of stroma is composed predominantly of delicate stromal cell processes. (×2548.)

mesonephric duct and paramesonephric duct regression occurs between 13 and 14 days.

The paramesonephric and mesonephric ducts could be readily differentiated by their position in the mesonephros; the paramesonephric duct is situated most laterally, between the overlying coelomic epithelium and the mesonephric duct. Besides positional differences in the mesonephros, the early developing gonoducts have certain light microscopic and ultrastructural characteristics that permit their identification. By light microscopy, cells of the paramesonephric ducts generally appear more pseudostratified than the mesonephric duct, an impression that is supported by the closely packed and crowded columnar cells with their nuclei at varying levels within the epithelium. As in the mouse,[1] mesonephric duct cells, in contrast, have relatively straight cell borders and less variation in nuclear position than their paramesonephric duct counterparts.

Both gonoducts show apical microvilli bordering the lumen; however, those of the paramesonephric ducts are generally more numerous and elongate, sometimes with terminal branching; the latter's appearance resembles that seen in the adult endometrium, fallopian tube, and endocervix, all of which are derived from the paramesonephric duct. Microvilli of the mesonephric duct cells are sparse, short, and stubby, a picture similar to that seen in the paratubal mesonephric ducts of the female adult.[7] Although both mesonephric ducts and paramesonephric ducts of the early embryo may produce cilia-like structures, they are rudimentary and the ciliary shafts lack the characteristic nine sets of peripheral double tubules surrounding a central pair of tubules. No well-formed ciliary bodies or cross-striated rootlets were identified. Ciliated cells found in paramesonephric-derived epithelia probably function in the movement of surface materials such as endometrial secretions. The absence of well-formed cilia in the developing gonoducts may reflect the lack of need for such locomotor ability at that particular stage of differentiation. Abundant cilia have been described in the tubules of the paratubal mesonephric remnants, but not in the mesonephric ducts of the female adult.[7]

The most striking light microscopic and ultrastruc-

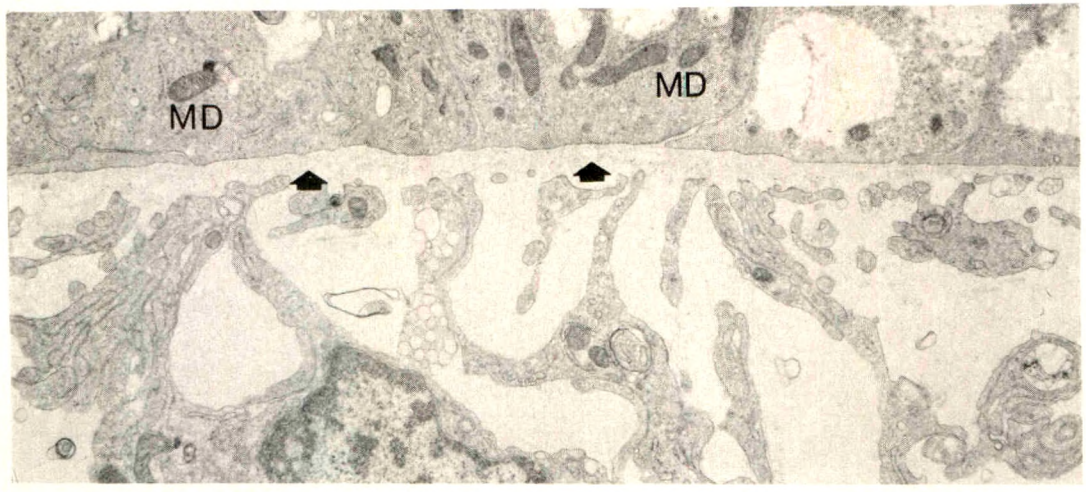


Fig. 7. Mesonephric duct. High magnification of epithelial-stromal interface shows elongated stromal cytoplasmic processes that make contact with basal lamina *(arrowheads)* of mesonephric duct *(MD)* cells. (× 10,200.)

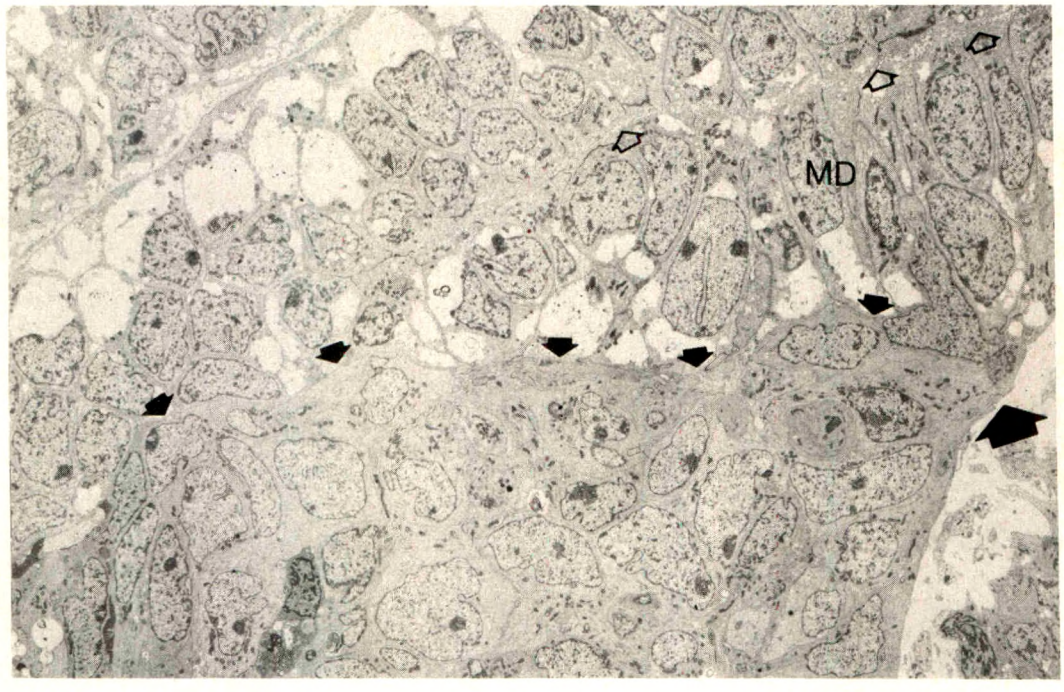


Fig. 8. Paramesonephric and mesonephric ducts. Advancing edge of paramesonephric duct *(large arrowhead)* composed of solid mass of cells without lumen formation growing adjacent to mesonephric duct cells *(MD)*. Paramesonephric-mesonephric duct junction is marked by *small solid arrowheads*. Slitlike lumen of mesonephric duct denoted by *open arrowheads*. (×2093.)

tural difference between the early gonoducts is the presence of abundant intracytoplasmic glycogen within the mesonephric duct cells. Glycogen most commonly forms a single, large subnuclear vacuole reminiscent of that seen in secretory endometrial glands of day 16 or 17. Similar aggregates of glycogen are rarely seen in cells of the paramesonephric duct. This is in contrast to the frequent and often prominent role of glycogen in the paramesonephric-derived tissues, especially the endometrial glands, of the mature female. In the adult endometrium, glycogen moves from a subnuclear to a supranuclear position and eventually is discharged into the lumen, presumably for nutritive purposes for the implanted conceptus. In the mesonephric ducts, conversely, glycogen infrequently assumes an apical position in the cytoplasm and has not been identified within

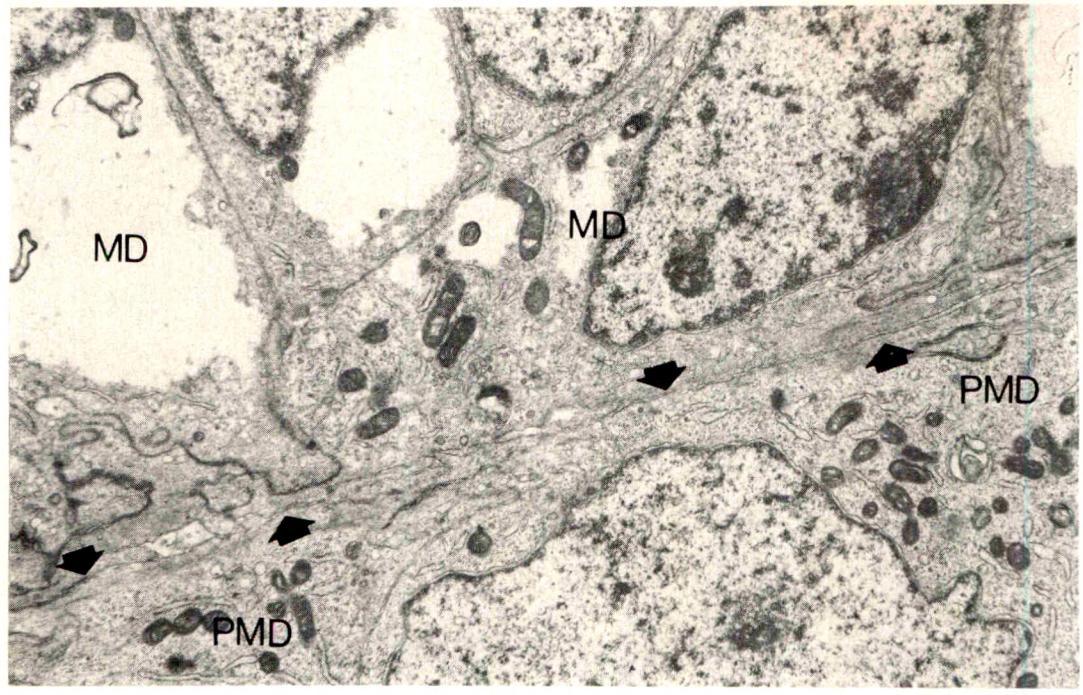


Fig. 9. Higher magnification of paramesonephric-mesonephric duct junction *(arrowheads)*. Neither basal lamina nor desmosomes are present between paramesonephric *(PMD)* and mesonephric *(MD)* duct cells. (×12,375.)

the lumen. Ultrastructural studies of the paratubal remnants in adult women[7] have demonstrated that the mesonephric ducts and tubules retain the ability to synthesize glycogen, as evidenced by the accumulation of glycogen particles in their cytoplasm. In the developing mesonephric ducts large aggregates of glycogen may be used for energy purposes rather than as a secretory product.

In cephalad sections, abundant mesenchyme clearly separates the gonaducts. More caudally, the ducts appear progressively closer until they are adjacent, without identifiable stroma or basal lamina between them. The latter finding, plus the clinical observation that paramesonephric, or müllerian, development is ablated if the mesonephric duct is missing, is responsible for the widely held belief that the paramesonephric duct uses the mesonephric duct as a "guidewire" in its descent to the urogenital sinus.

Few studies have dealt with the close anatomic relationship of the mesonephric and paramesonephric ducts. Gruenwald's early study of human and chick embryos[6] emphasized the influence of the mesonephric duct on the developing paramesonephric duct. Interruption of the mesonephric duct inhibited growth of the paramesonephric duct distal to that point. Developmental anomalies in man also support the hypothesis that a similar relationship exists between the paramesonephric and mesonephric ducts in human embryos.

The current ultrastructural study supports many of Gruenwald's light microscopic findings[6]; the caudal tip of the elongating paramesonephric duct is a solid wedge of cells forming a bulge on the lateral wall of the mesonephric duct, where it is closest to the coelomic epithelium. That this is the proliferating end of the paramesonephric duct is suggested by the numerous mitotic figures and the lack of a lumen at this stage of gonoductal development. Gruenwald interpreted the advancing wedge of paramesonephric duct cells to be within the basement membrane of the mesonephric duct. In our experience the basal lamina of the medial, or anticoelomic, aspect of the mesonephric duct is intact and continuous; however, the basal lamina appears to be interrupted at the most caudal point where the advancing paramesonephric duct cells contact the basal aspect of the mesonephric duct cells. No basal lamina was identified around the solid cord of paramesonephric duct cells, nor was basal lamina found between the two types of ductal cells in the region of the contiguous mesonephric and paramesonephric ducts.

Some early workers stressed, on the basis of light microscopic evidence, that the paramesonephric (müllerian) wedge splits off from the mesonephric duct. However, our findings show both to retain their histologic and ultrastructural identity throughout their length, including the area of apposition, which supports the separate origin of the two gonoducts.

Cunha and Lung[8, 9] have shown that normal development of the uterus and vagina depends on interactions between the epithelium and stroma of those tissues that are ultimately derived from the paramesonephric duct. The unusual relationship between the mesonephric and paramesonephric ducts and their periductal stroma suggests that epithelial-stromal interaction is important in the morphogenesis, and possibly maintenance, of both. Our ultrastructural findings suggest that the gonaducts have different morphologic patterns of epithelial-stromal contact. Intimate contacts between the stromal cell processes and the basal lamina of the mesonephric duct may provide an avenue for transfer of inductive substances to the basal lamina. The stromal cell processes are numerous so that the basal lamina of the mesonephric duct is virtually covered by them, resulting in a large area of stromal cell–basal lamina contact. The presence of large and prominent Golgi complexes polarized to the epithelial side of stromal cells indicates that they elaborate a secretory product, perhaps an inductive molecule. Pinocytotic vesicles located on the basal epithelial surface, similar to those described by Cunha and Lung[8] in the developing vagina, may represent endocytosis of the inductive material elaborated by stromal cells. Morphometric studies of the vesicles containing basal lamina—like material indicate that they originate from basal membrane and undergo degradation as they move into the cell.

In the paramesonephric duct, epithelial-mesenchymal contact is characterized by close proximity of stromal cell bodies to the base of the paramesonephric duct cells but without the interposition of delicate and elongate cell processes. The incomplete or absent basal lamina in these areas of intimate contact may facilitate the intercellular movement of inductive molecules. In such foci, epithelial cell processes extend into the stromal compartment and make physical contact with mesenchymal cell membranes. This phenomenon resembles that of the embryonic male rat paramesonephric duct[2] where the contact between epithelial and mesenchymal cells was associated with stripping of the basal lamina as a major event in early paramesonephric (müllerian) duct regression. The occurrence of a similar but focal phenomenon in the early paramesonephric duct suggests that close epithelial-mesenchymal cellular contact may occur focally to dissolve the basal lamina and facilitate intercellular transport. The bulk of the subepithelial stromal cell cytoplasm, including most of the organelles, is polarized to the ductal side of the cell. Therefore the Golgi apparatus, presumably the site of vesicle formation, is closest to the point of egress, or exocytosis, of its secretion. That actual transfer of molecules occurs from the mesenchymal to the epithelial compartment is suggested by the close fine structural similarity of the vesicles in both types of cells.

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Activation of protein kinase C stimulates collagenase production by cultured cells of the cervix of the pregnant guinea pig

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OBJECTIVE: Dilatation of the uterine cervix at parturition is achieved by an estrogen-induced, collagenase-mediated degradation of type I collagen in the cervix. The objective was to test the hypothesis that collagenase production in the cervix of pregnant guinea pig in culture is mediated by activation of protein kinase C.
STUDY DESIGN: The effects of 17β-estradiol, prostaglandin $F_{2\alpha}$, or activation of protein kinase C by phorbol-12-myristate-13-acetate, 1-stearoyl-2-arachidonyl-*sn*-glycerol and phospholipase C on collagenase production was studied with primary monolayer cultures of cervical cells from Hartley guinea pigs at 50 days' gestation. Results are analyzed for statistical significance with analysis of variance.
RESULTS: Collagenase production is increased twofold to threefold by 17β-estradiol, prostaglandin $F_{2\alpha}$, or activation of protein kinase C. The observed stimulation of collagenase production by 17β-estradiol and prostaglandin $F_{2\alpha}$ was blocked by the protein kinase C inhibitor 1-(5-isoquinoline sulfonyl) 2-methylpiperazine dihydrochloride.
CONCLUSION: Collagenase production in cultured cervical cells of pregnant guinea pig is stimulated by activation of protein kinase C. (Am J Obstet Gynecol 1992;167:194-200.)

Key words: Cervical dilatation, collagenase, estrogens, prostaglandins, protein kinase C

Interstitial collagenase is a Ca^{2+} and Zn^{2+}-dependent metalloprotease, the action of which is an essential component of the overall metabolism of fibrillar collagen in the extracellular matrix. Collagenase cleaves collagen types I, II, and III, the predominant collagens of mammalian tissues. Its sole action is to cleave collagen by hydrolyzing the peptide bonds between Gly-Leu in the α_2-chain and Gly-Ile in the α_1-chain of type I collagen at a single locus in the native triple helical domain.[1] Once the collagen molecule is cleaved into two fragments (¾ length fragment TC^A and ¼ length fragment TC^B), the fragments denature and are further cleaved by gelatinase, PZ-peptidase, and dinitrophenyl-peptidase, followed by phagocytosis and further digestion by lysosomal enzymes.[1, 2]

Collagen degradation is observed in biologic processes characterized by remodeling of connective tissue, such as bone resorption, wound healing, embryogenesis, postpartum uterine involution, and ovulation and in diseases such as osteoarthritis, rheumatoid arthritis, corneal ulcers, epidermolysis bullosa, periodontal disease, and tumor invasion.[2] Rapid collagen degradation is an underlying feature of cervical dilatation at parturition in both human and guinea pig.[3-7]

We have been interested in the mechanism of cervical dilatation at parturition. We have previously shown that cervical dilatation is accompanied by a considerable increase in extractable collagenase in the uterine cervix in women and in the guinea pig at parturition.[3, 7] High levels of circulating collagenase are also present in women in premature labor.[8] Such studies have indicated that collagenase probably plays an important role in the rapid collagen degradation observed in normal parturition and in the pathophysiology of premature labor.

We have chosen primary monolayer cultures of cells isolated from the cervices of pregnant guinea pigs at 50 days' gestation to study the mechanism of signal transduction of collagenase production for the following reasons: (1) there is an inadequate supply of cervical tissue from pregnant women, (2) cervical dilatation at parturition in both species is associated with a decrease in collagen (chemically and histologically) and a significant increase in extractable levels of collagenase in the cervix,[3, 4, 7] and (3) the guinea pig's reproductive system resembles that of women in that it has a similar ovu-

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lation cycle, ovulates spontaneously, and has an actively secreting corpus luteum, and most importantly continuation of pregnancy is not dependent on ovarian hormones after the first half of pregnancy.[9] These characteristics make the guinea pig an appropriate model for studies of human reproduction.[9]

Collagenase is synthesized and rapidly secreted by fibroblasts and chondrocytes.[1] Synthesis and secretion are stimulated by platelet-derived growth factor,[10] epidermal growth factor,[11] interleukin-1,[12] tumor necrosis factor,[13] and phorbol esters.[14] We have previously shown that collagenase messenger ribonucleic acid and collagenase activity are stimulated by human recombinant interleukin-1β and 17β-estradiol in monolayer cell cultures of cervixes of pregnant guinea pigs at 50 days gestation.[6] Furthermore, collagenase biosynthesis in culture is mediated by prostaglandins (PG) $F_{2\alpha}$ and E_2.[6] The mechanism of signal transduction of collagenase production in the guinea pig cervix is not known. This study examines the role of protein kinase C on collagenase production in the cervix of the pregnant guinea pig. Hydrolysis of endogenous phosphatidylinositol bisphosphate by phospholipase C results in the release of inositol triphosphate and diacylglycerol.[15] The release of inositol triphosphate and diacylglycerol results in increasing intracellular calcium concentration and activation of protein kinase C, respectively.[16] The present study extends our previous findings by providing evidence that activation of protein kinase C results in stimulation of collagenase production in cervical cell cultures of guinea pigs at 50 days' gestation.

Material and methods

Material. The following reagents were obtained from the Sigma Chemical Company, St. Louis: tamoxifen, $PGF_{2\alpha}$, 17β-estradiol, phorbol myristate acetate, 1-Stearoyl-2-arachidonyl-*sn*-glycerol, phospholipase C, bacterial collagenase type AI, deoxyribonuclease type 1, and *bis*-benzimide (Hoechst H33258). Trypsin 1:250 was obtained from Difco Laboratories, Detroit. 1-(5-Isoquinoline-sulfonyl)-2-methylpiperazine dihydrochloride (H-7) was obtained from Seikagaku America, St. Petersburg, Fla. Aquasol scintillation fluid and tritiated-methyl-thymidine were obtained from New England Nuclear, Dupont, Markham, Ontario.

Animals. Adult Hartley guinea pigs (Charles River, Montreal) were housed in guinea pig cages (three to five per cage) with a 12-hours-light and 12-hours-dark cycle and fed guinea pig chow and water as desired. Timed gestation was induced by mating animals within 12 hours of delivery. Pregnant animals at 50 days of gestation ($n = 10$) were anesthetized with intramuscular ketamine hydrochloride (50 mg/kg) and xylazine (3 mg/kg). After approximately 15 minutes they were killed by intracardiac exsanguination. With aseptic technique a midline laparotomy and a total abdominal hysterectomy were performed according to a protocol approved by the Royal Victoria Hospital Research Animal Care Sub-Committee.

Cervical cell isolation and culture. Cervices obtained from 10 pregnant guinea pigs at 50 days' gestation were cut into 2 to 4 mm pieces and digested with 0.05% trypsin 1:250/0.02% ethylenediaminetetraacetic acid (EDTA) in calcium and magnesium-free phosphate-buffered saline solution (for 15 minutes on ice, followed by digestion with bacterial collagenase type AI, 0.5 mg/ml) and deoxyribonucleas type I (0.05 mg/ml) in Dulbecco's modified Eagle medium for 4 to 6 hours at 37° C with gentle agitation. The digested mixed cervical cells were recovered by centrifugation at 365*g* for 10 minutes at room temperature every 1 to 2 hours followed by washing once with Hanks' balanced salt solution. The process was repeated until the tissue was completely digested (usually within 6 hours). The released cells were filtered twice through nylon monofilament with a pore size of 400 μmol/L (Swiss Nitex, Thompson, Montreal) and recovered by centrifugation at 365*g* for 10 minutes at room temperature. The cells were washed three times with Hanks' balanced salt solution followed by suspension in Dulbecco's modified Eagle medium containing heat-inactivated (60° C for 30 minutes) 10% fetal calf serum, penicillin G sodium (100 U/ml), streptomycin sulfate (100 μg/ml), and amphotericin B (1.25 μg/ml). Cell viability exceeded 90% as determined by the trypan blue exclusion test (approximately 3×10^6 cells per cervix). For each experiment three to four pregnant guinea pigs at 50 days of gestation were used. Cells were counted with a hemocytometer slide. Cells were plated in Limbro plates containing 96 flat-bottom wells, (Flow Laboratories, McLean, Va.) at 1×10^5 cells per well in 300 μl medium and incubated in a humidified atmosphere of 5% carbon dioxide in air at 37° C. After 3 days almost all cells had adhered. The medium and unattached cells were removed and replaced with fresh medium. Cells were then grown to confluency (usually within 3 days). Immediately before analysis of collagenase production, the cells were washed once in Hanks' balanced salt solution, followed by the addition of different concentrations of the chemicals and hormones to be tested in serum-free Dulbecco's modified Eagle medium containing penicillin, streptomycin, and amphotericin B at the concentrations described above with appropriate controls containing ethanol, which was used as the solvent at 0.1% to 0.3% final concentration of ethanol in Dulbecco's modified Eagle medium. After a further 72 hours culture media were collected and frozen at −20° C until assayed for collagenase. The cells were collected after a brief exposure to 100 μl per well of 0.05% trypsin and 0.02% EDTA in calcium and mag-

Table I. Tritiated methyl thymidine incorporation

	Incorporation	
	Counts/min (mean ± SEM)	*%*
Control Dulbecco's minimum essential medium	62,753 ± 3,393	5.76
Phorbol-12-myristate-13-acetate (10^{-8} mol/L)	57,025 ± 1,204	5.24
1-Stearoyl-2-arachidonyl-*sn*-glycerol (10^{-7} mol/L)	55,025 ± 7,552	5.11
Phospholipase C (0.1 U/ml)	43,849 ± 8,425	4.03
17β-Estradiol (10^{-7} mol/L)	66,683 ± 5,859	6.12
$PGF_{2\alpha}$ (10^{-6} mol/L)	51,080 ± 8,862	4.69

Tritiated methyl thymidine added to each well contained 1.1×10^6 counts/min. Each value gives the mean value ± SEM for quadruplicate wells. There were no significant differences.

nesium-free phosphate buffered saline solution (5 to 10 minutes, 37° C). Representative wells were counted with a hemocytometer slide. Cells from the remaining wells were frozen at −20° C for deoxyribonucleic acid (DNA) determinations.

Collagenase assay. Acid-soluble collagen was purified from the skins of adult female Sprague-Dawley rats, and the collagenase substrate tritiated telopeptide-free collagen type I was prepared as previously described.[6] Collagenase was assayed with the specific collagenase method described by Dean and Woessner[17] with some modifications. Briefly, enzyme samples (up to 100 μl in assay buffer) were incubated with 10 μl of tritiated telopeptide-free collagen substrate (specific activity 3.1×10^6 counts/min/mg collagen at 2.24 mg/ml, in 50 mmol/L Tris hydrochloride, pH 7.6, 0.3 mol/L sodium chloride) in 1.5 ml microfuge tubes (39 × 10 mm, Sarstedt, Germany). Aminophenylmercuric acetate (0.5 mmol/L) was added to activate procollagenase. 1,10-Phenanthroline (1 mmol/L) was added to inhibit collagenase and served as a blank control. After 18 to 48 hours of incubation at 30° C, the reaction was terminated by the addition of EDTA, to a final concentration of 40 mmol/L. Collagenase cleavage products (TC^A, TC^B) were further digested by trypsin and chymotrypsin in the presence of type I collagen as a carrier protein for 2 hours at 30° C. The undigested tritiated telopeptide-free substrate was precipitated in 10% trichloroacetic acid at 0° C for 30 minutes in the presence of 250 μg bovine serum albumin (10 mg/ml assay buffer) followed by centrifugation in a fixed speed Eppendorf centrifuge 5413 (Brinkman, Westbury, N.Y.) for 15 minutes. A 100 μl aliquot of the supernatant was mixed with 5 ml Aquasol scintillation fluid and counted in a Beckman scintillation spectrometer (Beckman, Palo Alto, Calif.). Mixing of these volumes resulted in minimal quenching of radioactivity (data not shown). The percent digestion was calculated as the total counts per minute in the supernatant minus counts per minute in the 1,10-phenanthroline blanks divided by original counts in the tritiated telopeptide-free substrate (in 100 μl of 10% trichloroacetic acid in assay buffer) × 100. One unit of collagenase is defined as the amount of collagenase that digests 1 μg of tritiated telopeptide-free collagen type I in 1 minute at 30° C.

Inactivation of tissue inhibitor of metalloproteinase. To prevent the inactivation of activated collagenase by tissue inhibitor of metalloproteinase, the latter was inactivated by reduction of its six disulfide bridges in 2 mmol/L dithiothrietol at 37° C for 30 minutes. To prevent spontaneous reactivation of tissue inhibitor of metalloproteinase, the reduced form was alkylated with 5 mmol/L iodoacetamide at 37° C for 30 minutes. The samples were dialyzed against assay buffer for 18 hours at 4° C, then assayed for collagenase. This treatment increases the detection of collagenase and does not interfere with collagenase activity.[6, 7]

DNA determination. DNA was determined as previously described.[6] Briefly, cervical tissues were digested with proteinase K (0.5 mg/ml) for 24 hours at 56° C in 0.1 mol/L sodium phosphate buffer, pH 6.5, containing 0.01% EDTA. DNA standard curves were prepared from calf thymus DNA (0.1 to 1.0 μg) in 0.1 mol/L phosphate and EDTA buffer. To 100 μl of tissue digest was added 2 ml of a solution containing 1.0 μg/ml *bis*-benzimide in 0.05 mol/L sodium phosphate, pH 7.4, containing 2 mol/L sodium chloride. Fluorescence was recorded with an excitation at 356 nm and emission at 458 nm with a fluorescence spectrophotometer (model 650-IOS, Perkin-Elmer Corporation, Norwalk, Conn.).

Tritiated thymidine incorporation. Primary monolayer cell cultures of cervices from guinea pigs at 50 day's gestation were maintained in 96-well plates in Dulbecco's modified Eagle medium with 10% fetal calf serum until confluency, as described above. The cells were washed twice in Hanks' balanced salt solution followed by the addition of 75 μl of serum-free Dulbecco's modified Eagle medium (in the presence or absence of hormones and chemicals to be tested). Twenty-five microliters of serum-free Dulbecco's modified Eagle medium containing 1 μCi of tritiated methyl thymidine (6.7 Ci/mmol) were added during the last 18 hours in

culture. Cells were incubated in an atmosphere of 5% carbon dioxide in air at 37° C. Culture media were removed, and cells were suspended by brief exposure to 100 μl per well of 0.05% trypsin and 0.02% EDTA in calcium-and magnesium-free phosphate-buffered salt solution for 5 minutes at 37° C. Cells were harvested on filter mats with a cell harvester (Titertek Cell Harvester, Skraton Inc., Sterling, Va.). Filters were added to 10 ml Aquasol scintillation fluid and counted in a Beckman scintillation spectrometer.

Statistical analyses. Results are expressed as the mean ± 1 SEM unless otherwise indicated. Statistical analyses were performed by analysis of variance for multiple observations with RS/1 Statistical Tools (BBN Software Products Corporation, Cambridge, Mass.). Statistical differences were determined with Bonferroni simultaneous confidence intervals for all comparisons, when applicable. Differences between groups were considered significant when $p \leq 0.05$. Dose-response curve fitting was obtained with DeltaGraph version 1.5 with polynomial of degree setting at 3 (DeltaPoint, Inc., Monterey, Calif.).

Results

Effects of phorbol myristate acetate, diacylglycerol, phospholipase C, 17β-estradiol, and $PGF_{2\alpha}$ on DNA replications. Confluent cells were exposed to these chemicals and hormones in serum-free Dulbecco's modified Eagle medium in the presence of tritiated methyl thymidine. There was no significant effect on tritiated thymidine incorporation into DNA when compared with control under these conditions (Table I).

Stimulation of procollagenase by known activators of protein kinase C. The addition of the tumor promoter phorbol-12-myristate-13-acetate (Fig. 1, *A*) or the diacylglycerol 1-stearoyl-2-arachidonyl-*sn*-glycerol (Fig. 1, *B*) resulted in a twofold stimulation of procollagenase production. Each of these activators, at all concentrations tested, resulted in an increase in procollagenase production. Dose-response effects were observed with a maximum response at 10^{-8} mol/L for phorbol-12-myristate-13-acetate and 10^{-7} mol/L for 1-stearoyl-2-arachidonyl-*sn*-glycerol. Similarly, the addition of bacterial phospholipase C (0.01 to 10 U/ml Dulbecco's modified Eagle medium) also resulted in a threefold stimulation of procollagenase production with a maximum response at 0.1 U/ml (Fig. 2).

Evidence for role of protein kinase C in mediating procollagenase stimulation of prostaglandins and estrogen. $PGF_{2\alpha}$ (10^{-6} mol/L) (Fig. 3, *A*) and 17β-estradiol (10^{-7} mol/L) (Fig. 3, *B*) stimulate the production of procollagenase in culture. The observed stimulation by both hormones was completely blocked by 1-(5-isoquinoline sulfonyl)-2-methylpiperazine dihydrochloride, H-7, an inhibitor of protein kinase C.[18] The stimulatory effect of 17β-estradiol but not that of $PGF_{2\alpha}$ was completely abolished by the estrogen antagonist tamoxifen.

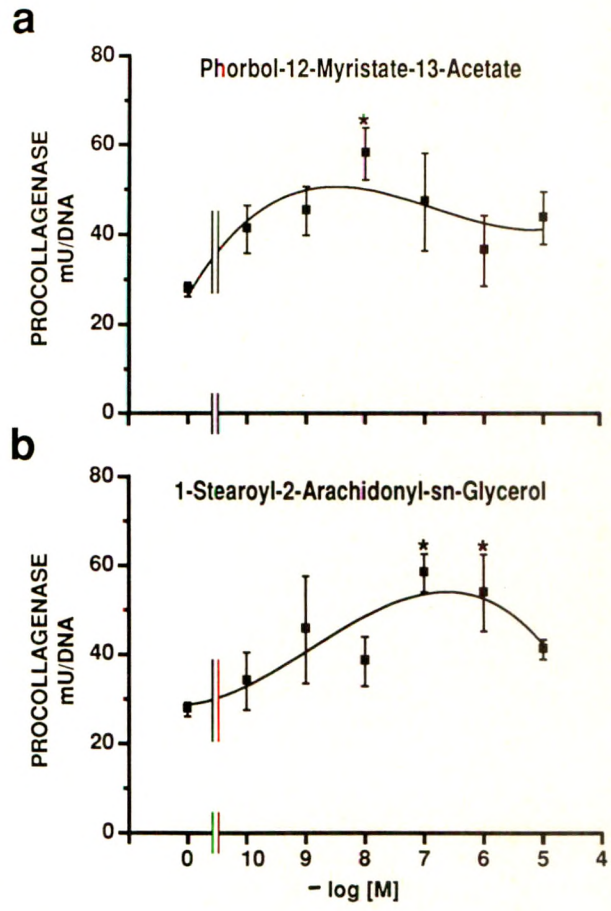


Fig. 1. Effects of activation of protein kinase C by phorbol-12-myristate-13-acetate **(a)** and by diacylglycerol 1-stearoyl-2-arachidonyl-sn-glycerol **(b)** on procollagenase production from primary monolayer cervical cell cultures of four pregnant guinea pigs at 50 days' gestation. Culture medium from each well was reduced with dithiothreitol, alkylated with iodoacetamide, dialyzed, and assayed for collagenase in duplicate in the presence of aminophenylmercuric acetate (0.5 mmol/L, added to activate procollagenase). Samples assayed for collagenase in presence of 1,10-phenanthroline (1 mmol/L, added to inhibit collagenase) served as blank controls. Mean ± SEM. Each point determination represents mean of quadruplicate wells assayed for collagenase. *–Log[M],* Negative logarithm of molar concentration; *asterisk,* $p < 0.05$ versus control (analysis of variance).

Comment

Interstitial collagenase is believed to play an important role in the cleavage of collagen during cervical dilatation at parturition in the human.[3] We have shown that the pregnant guinea pig can be used to study the regulation of this process.[4-7] In this study we have extended our previous findings to examine the mechanism of signal transduction of collagenase production in cultured cervical cells of the guinea pig at 50 days'

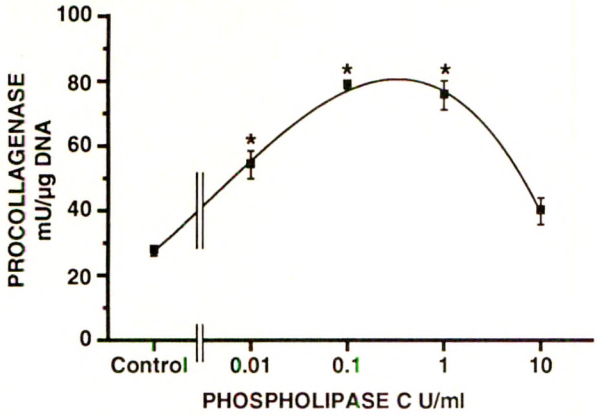


Fig. 2. Effects of hydrolysis of endogenous phosphatidylinositol bisphosphate by bacterial phospholipase C on procollagenase production from primary monolayer cervical cell cultures of three pregnant guinea pigs at 50 days' gestation. Culture medium from each well was reduced with dithiothreitol, alkylated with iodoacetamide, dialyzed, and assayed for collagenase in duplicate in the presence of aminophenylmercuric acetate (0.5 mmol/L, added to activate procollagenase). Samples assayed for collagenase in presence of 1,10-phenanthroline (1 mmol/L, added to inhbit collagenase) served as blank controls. Mean ± SEM. Each point determination represents mean of quadruplicate wells. *Asterisk*, $p < 0.05$ versus control (analysis of variance).

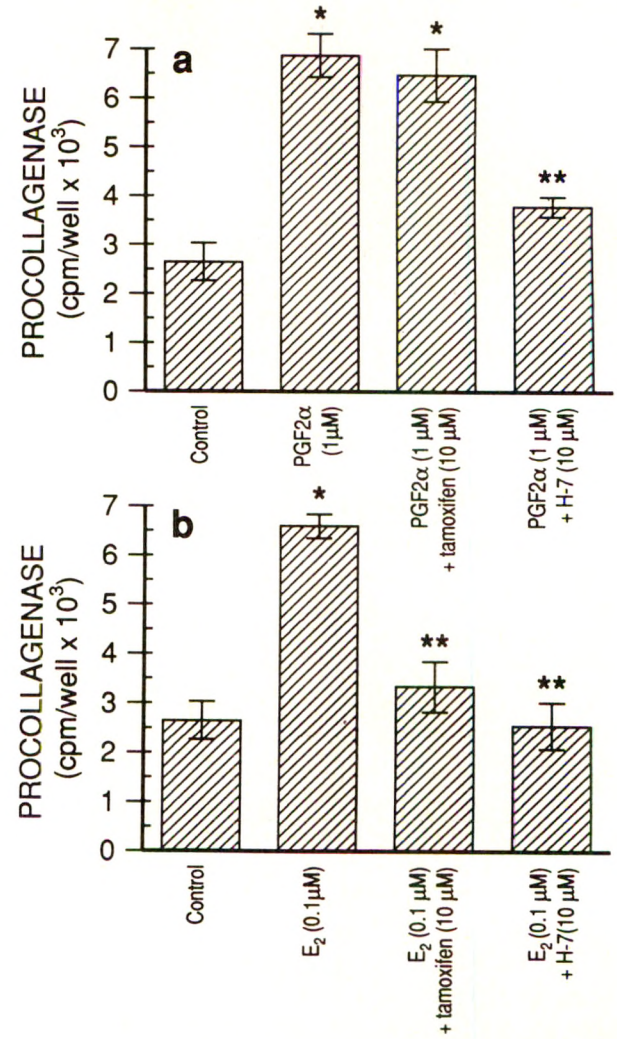


Fig. 3. Effects of $PGF_{2\alpha}$ **(a)** and 17β-estradiol *(E_2)* **(b)** as modified by estrogen antagonist tamoxifen and protein kinase C inhibitor 1-(5-isoquinoline sulfonyl)-2-methylpiperazine dihydrochloride *(H-7)* on procollagenase production in primary monolayer cervical cell cultures of three pregnant guinea pigs at 50 days' gestation. Each point determination represents mean of quadruplicate wells assayed for collagenase in duplicate after reduction with dithiothreitol, alkylation with iodoacetamide, and dialysis in presence of 0.5 mmol/L aminophenylmercuric acetate. *Asterisk*, $p < 0.05$ versus control; *asterisks*, $p < 0.05$ versus $PGF_{2\alpha}$ **(a)** and 17β-estradiol **(b)** (analysis of variance).

gestation. Collagenase is not stored in fibroblasts, and biosynthesis is accompanied by prompt secretion of the enzyme into the extracellular matrix.[1] Therefore secretion of the enzyme is a measure of collagenase biosynthesis.[6] Ordinarily, activated procollagenase is inactivated by available tissue inhibitor of metalloproteinase as soon as the enzyme is activated. With a recently developed sensitive and specific assay to detect latent procollagenase and a method to inactivate tissue inhibitor of metalloproteinase, it was possible to measure the total latent enzyme produced by these cells. It was also important to determine the mitogenic effect of the chemicals and hormones used in this study on cervical cells. As shown in Table I, there was no significant effect on cell division as determined by tritiated thymidine incorporation of any of the reagents tested compared with the controls when using confluent cultures and serum-free medium. The dissociation between mitogenic activity and protein synthesis under these conditions allowed us to investigate the effect of these reagents on procollagenase biosynthesis in culture.

The role of protein kinase C activation in procollagenase biosynthesis was studied. Hydrolysis of endogenous phosphatidylinositol bisphosphate by bacterial phospholipase C[15] and activation of protein kinase C by phorbol-12-myristate-13-acetate and diacylglycerol resulted in twofold to threefold stimulation of procollagenase production. These results indicate that production of procollagenase may be mediated by activation of endogenous phospholipase C resulting in hydrolysis of phosphatidylinositol biphosphate, production of diacylglycerol, and activation of protein kinase C. We have previously shown that 17β-estradiol stimulates procollagenase production in cultured cervical cells of guinea pigs at 50 days' gestation through a prostaglandin intermediate with an optimum response at 10^{-7} mol/L.[6] $PGF_{2\alpha}$ also stimulates procollagenase production with an optimum response at 10^{-6} mol/L.[6] In this study the stimulatory effect of 17β-estradiol and not that of $PGF_{2\alpha}$ was completely blocked by the estrogen antagonist tamoxifen, indicating a specific effect of estrogen. The observed stimulatory ef-

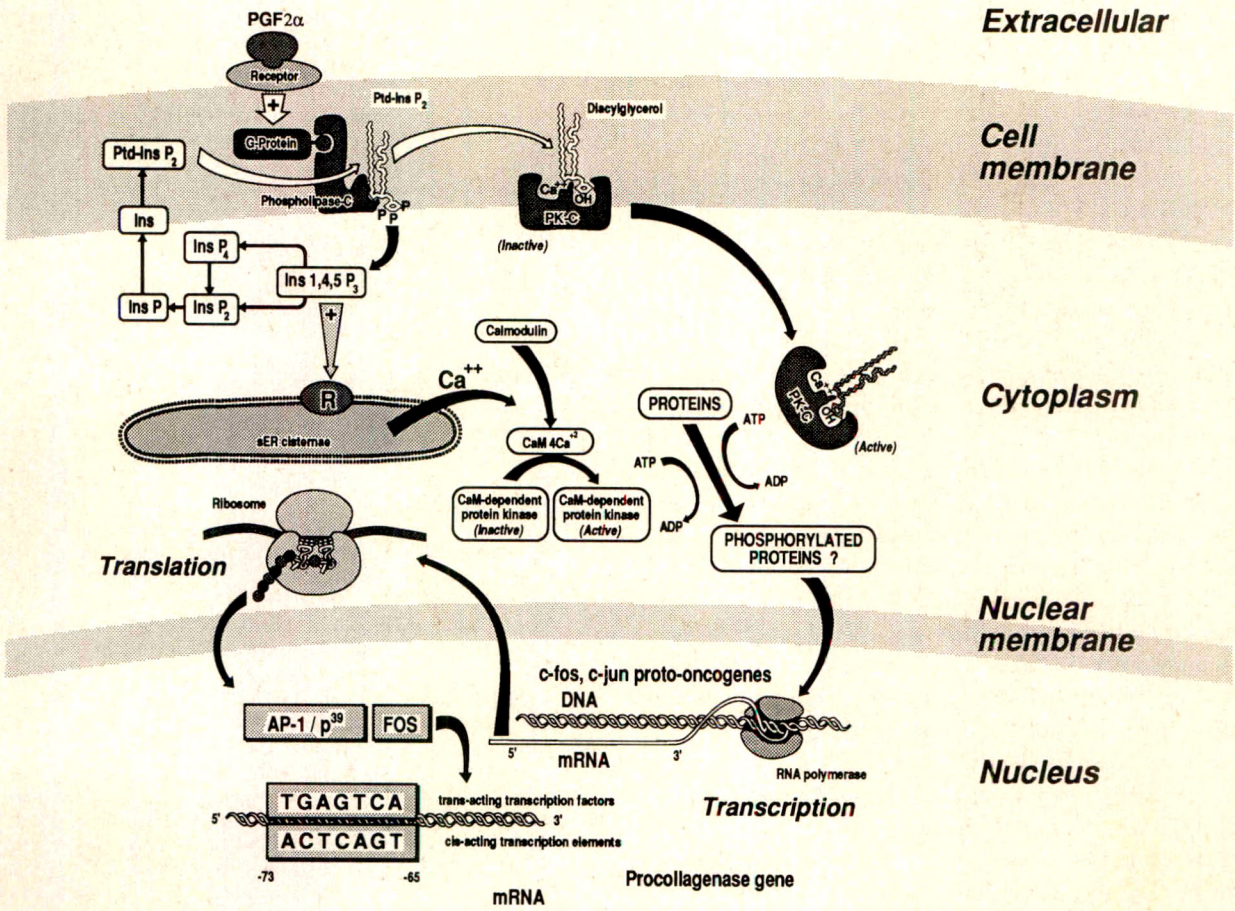


Fig. 4. Schematic presentation of possible mechanism of signal transduction of stimulation of procollagenase production by $PGF_{2\alpha}$. *PK-C,* Protein kinase C; *ptd-Ins P_2,* phosphatidylinositol bisphosphate; *sER,* smooth endoplasmic reticulum; *CaM $4Ca^{2+}$,* calcium-activated calmodulin; *AP-1/P39,* protein product of c-*jun* protooncogene; *FOS,* protein product of c-*fos* protooncogene. (See text for details).

fects of 17β-estradiol and $PGF_{2\alpha}$ were completely blocked by 1-(5-isoquinoline sulfonyl)-2-methylpiperazine dihydrochloride, a protein kinase C inhibitor[18] providing additional support for activation of protein kinase C in collagenase production. These findings are further supported by other reports. Estradiol-17β has been shown to stimulate renal PGE_2 production.[19] PGE_2 is converted to $PGF_{2\alpha}$ by PGE_2 9-ketoreductase that is widely distributed in several tissues.[20] Prostaglandin $F_{2\alpha}$ activates endogenous phospholipase C, resulting in hydrolysis of phosphatidylinositol bisphosphate and activation of protein kinase C.[21]

Other published evidence supports a role for protein kinase C activation in collagenase production. Phorbol myristate acetate, which mimics the effect of diacylglycerol and activates protein kinase C, is a potent stimulator of rabbit synovial cells to produce collagenase in culture.[21] Tumor necrosis factor-α also activates transcription of procollagenase messenger ribonucleic acid by a protein kinase C–mediated activation of the protooncogenes c-*fos* and c-*jun*.[13] The *trans*-acting transcription proteins of protooncogene products (AP1/P39 and FOS proteins) act on the *cis*-acting transcription elements TGAGTCA at positions −73 to −65 upstream of the procollagenase gene, resulting in its activation by a protein kinase C pathway.[13] Such studies provide strong evidence for $PGF_{2\alpha}$-induced, protein kinase–mediated activation of collagenase production. Although the effect of $PGF_{2\alpha}$ on c-*jun* and c-*fos* is not known, the most likely outcome of activation of protein kinase C by $PGF_{2\alpha}$ is an immediate activation of these protooncogenes, resulting in activation of collagenase gene. A schematic presentation of our current state of knowledge on possible $PGF_{2\alpha}$-mediated signal transduction pathways leading to an activation of collagenase gene is presented in Fig. 4.

In this study our finding of stimulation of collagenase production in cultured cervical cells of guinea pigs at 50 days' gestation by $PGF_{2\alpha}$ is in agreement with our previously published studies[5-7] and those of others.[22] With regard to the stimulatory effect of 17β-estradiol on collagenase production, findings from this study are in agreement with our previously published studies with cultured cervical cells of guinea pigs at 50 days' gestation and cultured cervical tissue of nonpregnant animals.[5-7] In the postpartum rat uterus administration

of 17β-estradiol resulted in inhibition of collagenase activity and retardation of postpartum involution of the uterus.[23] Jeffrey et al.,[24] on the other hand, have failed to demonstrate any significant effect on collagenase production by 17β-estradiol in organ culture of the postpartum rat uterus. The apparent discrepancies in the effects of 17β-estradiol on collagenase production may be due to one or more of the following: (1) different responsiveness of two different organs (i.e., uterus and cervix), (2) variation in uptake and metabolism of the administered hormones in vivo and in vitro, and (3) postpartum involuting rat uterus may respond differently from the cervix of the guinea pig at 50 days' gestation.

The finding that bacterial phospholipase C stimulates cervical collagenase production, a key enzyme in cervical dilatation at parturition, is of clinical significance in understanding the pathophysiologic parameters of preterm labor. There is a significant body of evidence suggesting a role for infection-mediated products such as interleukin-1β, platelet-activating factor, phospholipase A_2, phospholipase C, and tumor necrosis factor in the pathophysiologic features of preterm labor.[25] It is possible to speculate that phospholipase C and $PGF_{2\alpha}$ may lead to premature stimulation of cervical collagenase resulting in degradation of type I collagen, the main structural protein in the extracellular matrix of the cervix, and leading to preterm cervical dilatation. High levels of circulating collagenase are present in women at parturition and significantly higher levels are present in women in premature labor, suggesting a role for collagenase in the pathophysiologic characteristics of premature labor.[8]

In summary, collagenase production in cultured cervical cells of 50-day pregnant guinea pigs is stimulated by activation of protein kinase C. Furthermore, 17β-estradiol and $PGF_{2\alpha}$ stimulate collagenase production by activation of protein kinase C.

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Hydrogen peroxide and reoxygenation cause prostaglandin-mediated contraction of human placental arteries and veins

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OBJECTIVE: Because our previous studies in several vascular preparations suggest that posthypoxic reoxygenation elicits tone responses by generation of hydrogen peroxide we compared the actions of reoxygenation and hydrogen peroxide on isolated human placental arteries and veins.

STUDY DESIGN: Endothelium-intact and denuded arteries and veins (1 to 2 mm diameter, from normal term deliveries), incubated under 95% oxygen/5% carbon dioxide or 5% oxygen/5% carbon dioxide (balance nitrogen) and precontracted with 1 to 10 nmol/L U46619, were exposed to hypoxia (95% nitrogen/5% carbon dioxide, P_{O_2} 8 to 10 torr) followed by reoxygenation and to 1 to 100 μmol/L hydrogen peroxide in the presence and absence of the inhibitor of prostaglandin biosynthesis, 10 μmol/L indomethacin.

RESULTS: In both arteries and veins posthypoxic reoxygenation or exposure to hydrogen peroxide produced vascular contraction that was eliminated or reversed to a modest relaxation by indomethacin, consistent with mediation by prostaglandins. Hypoxia after incubation with 5% oxygen, but not 95% oxygen, caused a modest prostaglandin-independent relaxation. Removal of the endothelium did not alter any of these responses.

CONCLUSION: Placental arteries and veins show a similar prostaglandin-mediated contraction to hydrogen peroxide and posthypoxic reoxygenation, consistent with a possible involvement of hydrogen peroxide in the response to reoxygenation. (Am J Obstet Gynecol 1992;167:201-7.)

Key words: Endothelium, hydrogen peroxide, oxygen, placental vessels, prostaglandins

Our studies of a variety of vascular preparations have suggested that reoxygenation, with physiologic or hyperoxic oxygen tensions, after a brief period of hypoxia causes vascular responses that appear to involve the generation of hydrogen peroxide.[1-3] For example, in the calf pulmonary and coronary artery reoxygenation appears to produce a hydrogen peroxide–elicited relaxation caused by the activation of guanylate cyclase, resulting in production of the intracellular mediator guanosine cyclic-3′,5′-monophosphate.[1, 2] In the rat skeletal muscle microcirculation, the reoxygenation caused by the release of a brief (15 second) arteriolar occlusion or the administration of hydrogen peroxide causes vasodilation mediated by both the generation of dilator prostaglandins and by a prostaglandin-independent mechanism, which may also involve guanosine cyclic 3′,5′-monophosphate.[3] Therefore the endogenous generation of hydrogen peroxide could contribute to responses in other vascular preparations produced by reoxygenation after exposure to hypoxia.

Changes in oxygen tension have been shown to alter vascular tone of the umbilical artery in vitro in both lamb[4] and human.[5-7] In the lamb umbilical artery both low and high P_{O_2} values were found to cause contraction mediated by acetylcholine,[4] whereas in the human umbilical artery only an increase in P_{O_2} causes contraction, which is mediated by cyclooxygenase products.[7] The human umbilical vein, however, was found to lack this oxygen-induced mechanism.[5] Other studies suggest that oxygen induces thromboxane formation in human umbilical vessels.[8] Exposure of human umbilical artery to oxygen was found to inhibit prostaglandin I_2 formation,[9, 10] whereas hypoxia stimulated its production.[9] The production rate of thromboxane A_2, prostaglandin E_2, and $F_{2\alpha}$, on the other hand, is larger at increased oxygen tension.[9] Thus an increase in P_{O_2} causes a prostaglandin-mediated contraction of human umbilical artery; however, little is known about the effects of changes in P_{O_2} on tone in blood vessels present in the placental segment of the circulation.

In the present study we investigated whether re-

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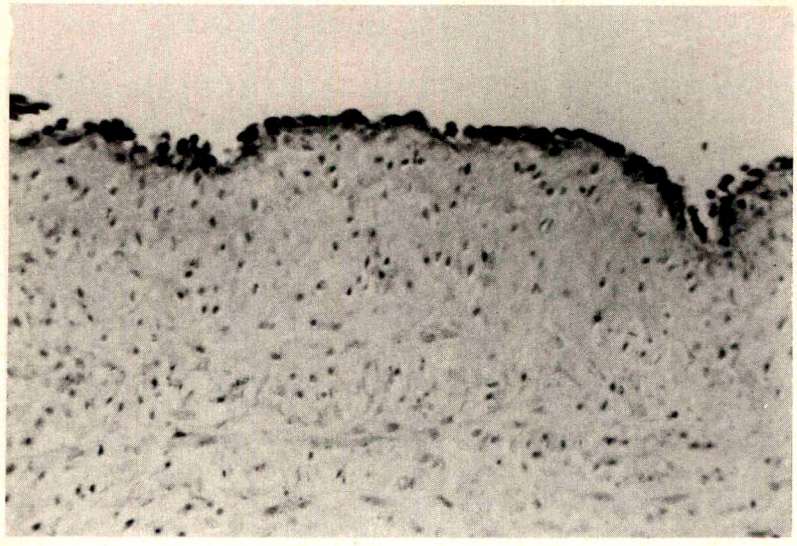


Fig. 1A. Section from endothelium-intact human placental vein immunostained for factor VIII antigen. Intact endothelial layer is clearly visible. (Original magnification ×60.)

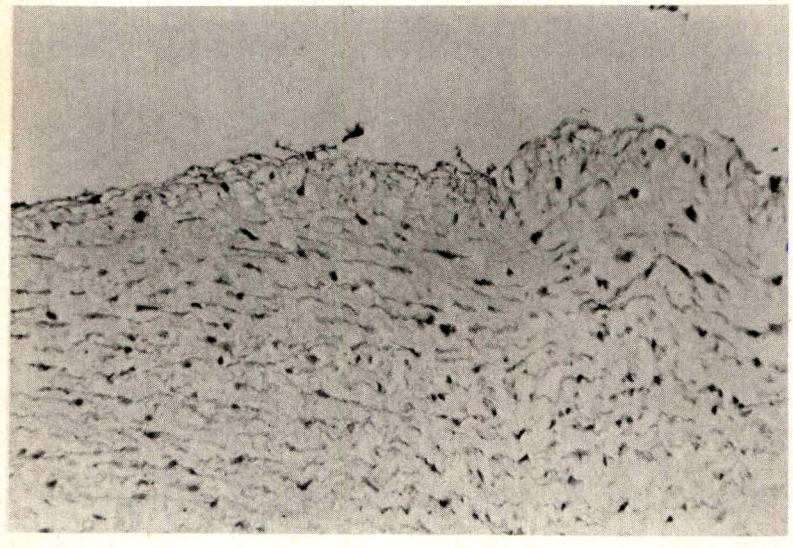


Fig. 1B. Section from endothelium-denuded human placental vein immunostained for factor VIII antigen. Endothelial layer here is absent. (Original magnification ×60.)

sponses to changes in P_{O_2} observed in the human umbilical artery were present in isolated human placental arteries and veins and whether there are similarities between the response to reoxygenation and to exposure to hydrogen peroxide. Because the endothelium could potentially influence vascular tone by the release of mediators, all studies with changes in P_{O_2} and hydrogen peroxide were conducted in the presence and absence of the endothelium. Because of the potential importance of prostaglandins in the oxygen-elicited responses of the human umbilical artery, the involvement of prostaglandins in all observed responses was examined by inhibition of their formation with indomethacin. In addition, responses were characterized both in the presence of an atmosphere of 95% (P_{O_2} ~ 500 to 600 torr) or 5% oxygen (P_{O_2} ~ 35 torr) for comparison with the hyperoxic environment used in previous studies on isolated vascular tissue or to approximate physiologic oxygen tensions in the placental venous circulation, respectively. A nitrogen atmosphere (P_{O_2} 8 to 10 torr) was used to generate hypoxia.

Methods

Experimental techniques. Placentas from uncomplicated term pregnancies were obtained immediately

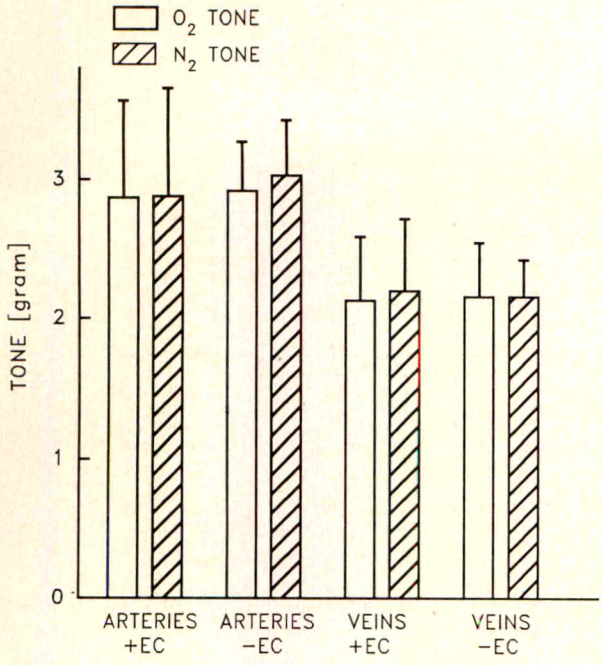


Fig. 2. Effect of brief hypoxia on tone generated by 1 to 10 nmol/L U46619 in human placental arteries (n = 5) and veins (n = 7), both endothelium intact *(+EC)* and denuded *(−EC)* after incubation under 95% oxygen *(O_2)*. Tone is increase in force produced by U46619, once tone has reached steady-state under oxygen and then after exposure to nitrogen *(N_2)* atmosphere. No alteration of tone was observed.

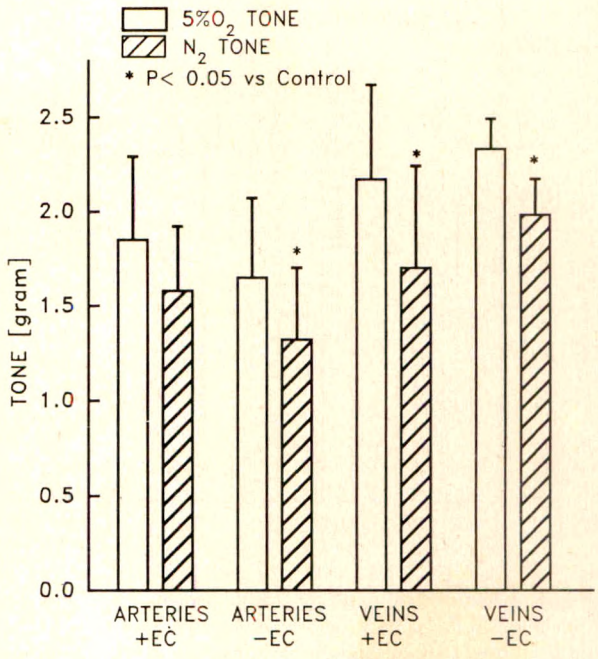


Fig. 3. Effect of brief hypoxia on tone generated by 1 to 10 nmol/L U46619 in human placental vessels (n = 6) after incubation under 5% oxygen. Modest relaxation was observed in all groups. Relaxation of endothelium-intact arteries did not reach statistical significance (p = 0.09) N_2, Nitrogen; *+EC*, Endothelium intact; *−EC*, endothelium denuded.

after vaginal delivery or cesarean section and placed in ice-cold saline solution. Placental arteries and veins were then dissected from the peripheral half of the fetal side of the chorionic plate within 1 hour of delivery and placed in ice-cold Krebs-bicarbonate buffer (pH 7.4) containing the following: sodium chloride, 118 mmol/L; potassium chloride, 4.7 mmol/L; calcium chloride, 1.5 mmol/L; sodium bicarbonate, 25 mmol/L; magnesium sulfate, 1.1 mmol/L; monopotassium phosphate, 1.2 mmol/L; and glucose, 5.6 mmol/L. The vessels were then cleaned carefully from surrounding tissue and cut, with a new scalpel, with extreme care to avoid damage to the endothelium into rings. The vessel rings, 1 to 2 mm in diameter and 2 mm in width, were mounted on wire hooks attached to Grass force displacement transducers (model FT03, Grass Instrument Co., Quincy, Mass.) for measurement of changes in isometric force. Vessels were incubated in 10 ml baths (Metro Scientific), which were temperature-controlled individually (37° C) in Krebs buffer gassed with either 95% oxygen–5% carbon dioxide or 5% oxygen–5% carbon dioxide–90% nitrogen. Tension was continuously adjusted during the equilibration period to 5 gm, which we found to be the optimal baseline force for observation of maximal contraction. The stretching of the vessels to this level of baseline force produced ~1 gm of spontaneous tone generation, on the basis of the maximal relaxation elicited by exposure to nitroglycerin. Changes in force were recorded on a Grass polygraph (model 7), and are reported as the increase in force above the 5 gm of combined passive and stretch-induced tone. Some of the rings were denuded of endothelium by gentle rubbing of the lumen of the vessel with the wooden handle of a cotton swab for about 40 seconds. After a 2-hour equilibration at the optimal passive tension, the vessels were depolarized with Krebs-bicarbonate containing potassium chloride (123 mmol/L) in place of sodium chloride. This treatment produces maximal contraction (~7 gm) and enhances the reproducibility of subsequent contractions. The vessels were then reequilibrated with Krebs-bicarbonate for 30 minutes before experiments were conducted.

The vessels were precontracted with 1 to 10 nmol/L U46619, tone was allowed to achieve a steady-state level, and the gas mixture bubbling the organ bath was then switched from 95% or 5% oxygen to 95% nitrogen (the three gas mixture contained 5% carbon dioxide, which maintained the pH at 7.4) for 5 to 7 minutes followed by rapid reoxygenation with the original gas mixture that was used before exposure to 95% nitrogen. This protocol was designed on the basis of obser-



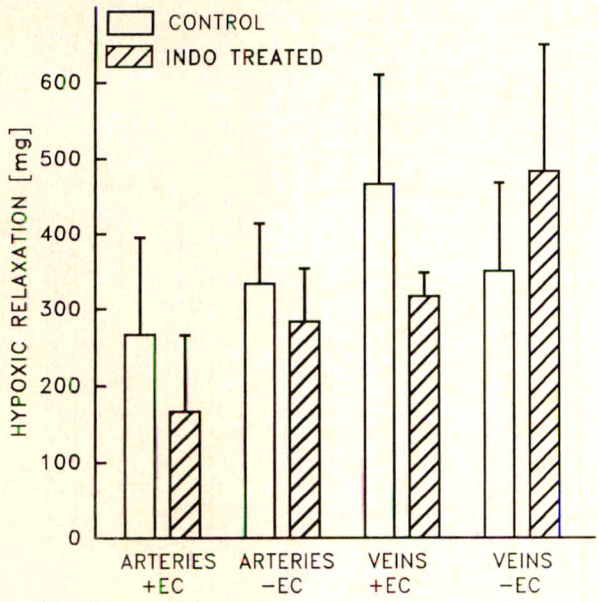


Fig. 4. Effect of indomethacin *(INDO)* treatment on hypoxic relaxation in human placental vessels ($n = 6$). No difference on level of relaxation between groups was observed, and indomethacin did not significantly alter relaxation to hypoxia in any group. *+EC,* Endothelium intact; *−EC,* endothelium denuded.

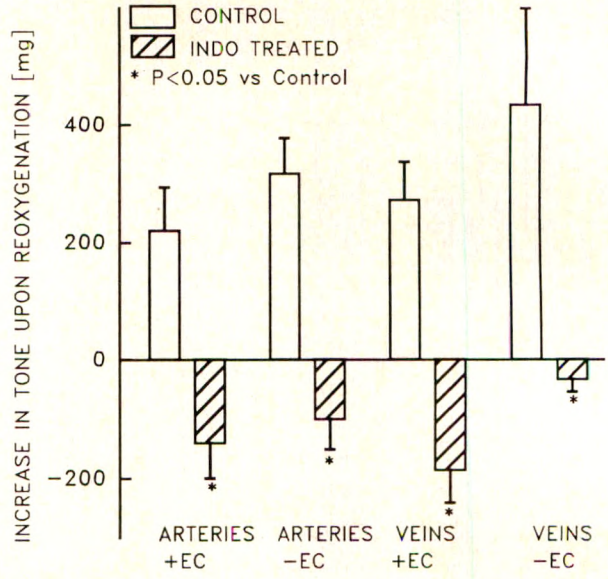


Fig. 5. Summary data showing contraction of human placental arteries ($n = 5$) and veins ($n = 7$) on reoxygenation with 95% oxygen and the effect of indomethacin *(INDO)* treatment on this contraction. No difference on level of contraction between groups was observed. In all groups contraction was reversed to modest relaxation by indomethacin. *+EC,* Endothelium intact; *−EC,* endothelium denuded.

vations from preliminary experiments, where it was found that agonist-induced tone of 1 o 4 gm and a 5-minute period of hypoxia were needed for optimal reproducible observation of the reoxygenation-elicited contraction, that U46619 produced contraction of these vessels that was very stable with time, and that changes in Po_2 produced by shifting the gas mixtures between 5% and 95% oxygen did not alter tone generation. Cumulative doses of hydrogen peroxide (1 to 100 μmol/L) were added to the organ baths when steady-state tone was reached again. A 2-minute period was allowed between doses, and after the highest dose the vessels were thoroughly washed with Krebs solution. After 30 minutes the protocol was repeated in a second experimental cycle. Some of the vessels were treated between the two cycles with 10 μmol/L indomethacin for 15 minutes and the others were used as time controls. Dilutions of U46619 and hydrogen peroxide were made in distilled-deionized water, and 10 μl aliquots were added to the 10 ml baths, as indicated. Indomethacin was dissolved in absolute ethyl alcohol and 10 μl from the solution was added to the bath. Vehicle control with 10 μl ethyl alcohol was done in the first cycle for all vessels in the indomethacin-treated group.

In most experiments both endothelium-intact and endothelium-denuded vessel rings were fixed in 10% buffered formalin, both before and after the experiment, and embedded in paraffin. Sections 5 μm thick were stained with the routine hematoxylin and eosin or immunostained for factor VIII antigen with a commercial staining kit (Dako Corp., Santa Barbara, Calif.) and with standard protocols, which we have routinely used for detection of various antigens.[11]

Material. U46619 was purchased from Cayman Chemical Company, Ann Arbor, Mich. Hydrogen peroxide (30%) and indomethacin were obtained from Sigma Chemical Company (St. Louis).

Statistical analysis. A paired Student t test was used to compare the level of tone or the change in tone between first and second cycle. A one-way analysis of variance with Duncans' multiple range test to determine significance between groups was used for multiple comparisons among different vessels to determine the effects of endothelium and comparisons between arteries versus veins on all responses to hypoxia, reoxygenation, or hydrogen peroxide. The accepted level of significance was $p < 0.05$. The number of experimental determinations in all cases is equal to the number of placentas from which a vessel ring was used for a treatment or control group. Data in the figures are depicted as means ± SE. In all experiments described, responses were found to be reproducible with time.

Results

Histologic examination of the vessels. Hematoxylin and eosin–stained sections clearly demonstrate an intact endothelial layer in control vessels, whereas that layer is almost totally (90% of intial surface) absent in

denuded vessels (not shown). Immunostaining for factor VIII antigen confirms the endothelial nature of the cells (Figs. 1A and 1B).

Effects of hypoxia. In the first group of experiments, conducted on placental arteries and veins gassed with 95% oxygen, exposure to a brief period of hypoxia (Po_2 8 to 10 torr) did not alter the level of tone in any group of vessels, as is shown in Fig. 2. Vessels incubated under 5% oxygen (Po_2 35 to 40 torr), however, underwent a relaxation of ~20% during the first 3 minutes of exposure to hypoxia (Fig. 3). This relaxation was statistically significant in all groups except the endothelium-intact arteries ($p = 0.09$). Similar results were also observed in the presence of indomethacin. Indomethacin did not significantly alter the magnitude of the hypoxic relaxation, as shown in Fig. 4.

Effect of reoxygenation. Reintroduction of 95% oxygen after 5 to 7 minutes of exposure to hypoxia elicited an increase in tone of about 15% to 20% in all vessels. This contraction started almost immediately after reoxygenation and reached maximum within 2 minutes. The duration of this contraction averaged 3 to 5 minutes and was followed by a return to the initial level of tone. Treatment with 10 μmol/L indomethacin eliminated the reoxygenation contraction or reversed it to a modest relaxation (Fig. 5). On the other hand, reoxygenation with 5% oxygen produced significant contraction only in the veins. The arteries showed a very modest increase in tone, which was not statistically significant (Fig. 6). As shown in Fig. 6, indomethacin treatment caused a complete elimination of this contraction, and in general a reversal to relaxation was observed.

Effects of hydrogen peroxide. Addition of 1 to 10 μmol/L hydrogen peroxide caused only a minor, nonsignificant increase in tone (not shown), whereas 0.1 mmol/L hydrogen peroxide elicited contraction in all vessels under both 95% and 5% oxygen (Figs. 7 and 8). There was no significant difference between arteries and veins in their response to hydrogen peroxide under 95% oxygen (Fig. 7), whereas under 5% oxygen the venous contraction to 0.1 mmol/L hydrogen peroxide was more than double that of the arteries (Fig. 8). Hydrogen peroxide addition after treatment with indomethacin, however, caused a modest relaxation in all vessels under both oxygen atmospheres (Figs. 7 and 8). Removal of the endothelium did not alter any of the responses to hypoxia, oxygen, or hydrogen peroxide as shown in Figs. 2 through 8.

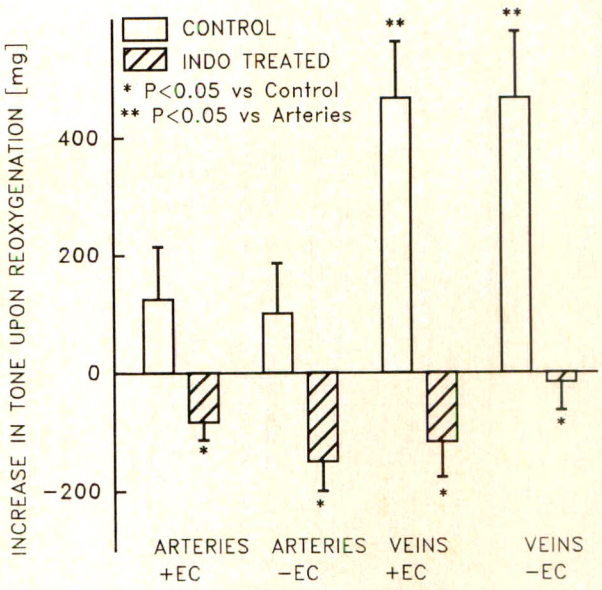


Fig. 6. Effect of reoxygenation with 5% oxygen on human placental vessels ($n = 6$). Veins contracted to significantly ($p < 0.05$) greater level than the arteries; indomethacin *(INDO)* eliminated this contraction and reversed it to modest relaxation in all groups. *+EC*, Endothelium intact; *−EC*, endothelium denuded.

Comment

Similar to findings in human umbilical artery,[5-7] this study shows that oxygen induces contraction in human placental arteries and veins. Responses to increments of Po_2 are not always maintained in human umbilical artery, and a hypoxic period before the introduction of oxygen is needed to observe a response to oxygen.[5-7] As previously described in the human umbilical artery,[5-7] the contraction to reoxygenation in the human placental vessels also seems to be mediated by cyclooxygenase products, because it was eliminated by indomethacin. This mechanism appears to be different from the oxygen-induced contraction in the sheep umbilical artery, where it was reported to be mediated by acetylcholine.[4] Unlike the human umbilical vein, which was reported to lack this oxygen-induced mechanism,[5] the human placental vein in this study was very sensitive to increases in Po_2.

The findings of this study in the human placental vessels is consistent with our previous studies in other vascular preparations[1-3] in that reoxygenation after a brief period of hypoxia leads to a response that is similar to that caused by exposure to hydrogen peroxide. In the human placental vessels hydrogen peroxide induces a prostaglandin-mediated contraction. Because reoxygenation produces a similar contraction, we hypothesize that an abrupt increase in Po_2 after exposure to hypoxia results in the generation of hydrogen peroxide. In our previous studies in other species we have shown that hydrogen peroxide produces tone responses by different mechanisms, including prostaglandins and guanosine cyclic 3′,5′-monophosphate.[1-3]

The observation that hypoxia did not alter tone of the human placental arteries and veins after incubation under 95% oxygen whereas those vessels equilibrated

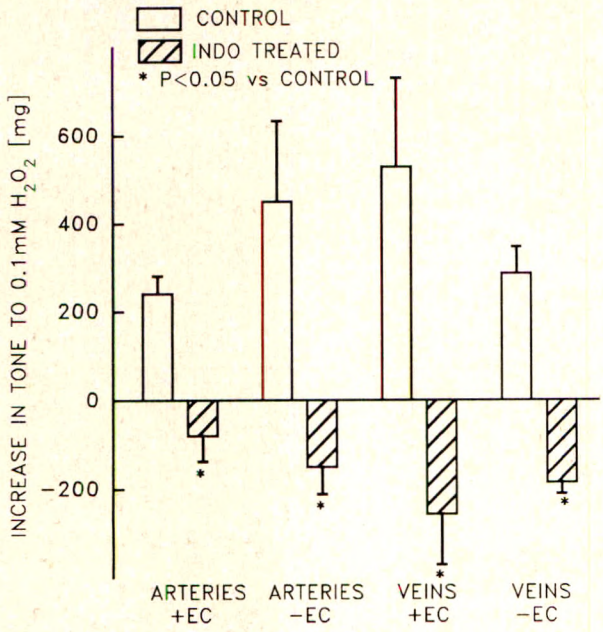


Fig. 7. Effect of topical application of 0.1 mmol/L hydrogen peroxide on human placental arteries (n = 6) and veins (n = 7) under 95% oxygen. No difference on level of contraction between groups was observed. In all groups contraction was reversed to modest relaxation by indomethacin *(INDO)*. *+EC*, Endothelium intact; *−EC*, endothelium denuded.

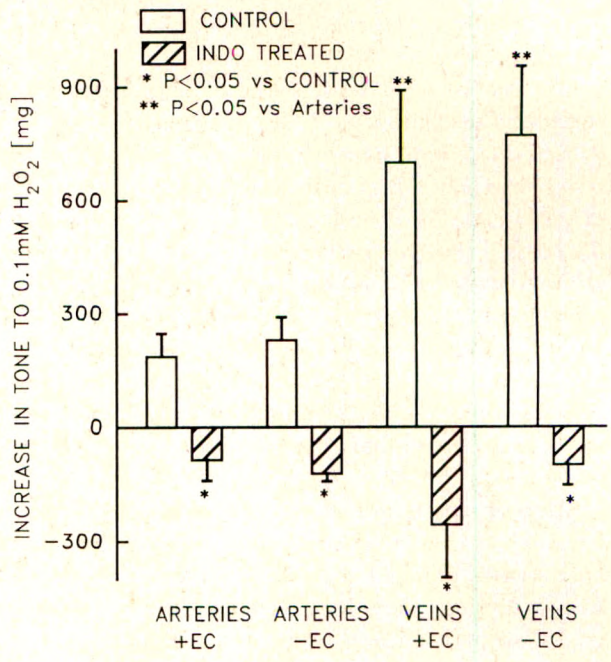


Fig. 8. Effect of 0.1 mmol/L hydrogen peroxide on human placental vessels (n = 7) under 5% oxygen. Veins contracted to significantly ($p < 0.05$) greater level than arteries. Indomethacin *(INDO)* reversed the contraction to modest relaxation. *+EC*, Endothelium intact; *−EC*, endothelium denuded.

under 5% oxygen, showed relaxation to hypoxia is similar to observations in rabbit arteries where chronic exposure to physiologic oxygen tension led to a change in the response to hypoxia.[12] This change in response was termed *adaptation*, a term used to describe previous observations that a prolonged exposure of isolated blood vessels to a certain normoxic or hypoxic oxygen tension resulted in a change in the tone response elicited by exposure to severe hypoxia, followed by reoxygenation to the original oxygen atmosphere. This is similar to the expression of the relaxation caused by exposure to nitrogen atmosphere after prolonged incubation with 5% oxygen, which was not observed in the experiments conducted with 95% oxygen. The observed hypoxic relaxation of human placental vessels in this study does not appear to be mediated by prostaglandins, because it was not significantly changed by indomethacin. However, the mechanism for these responses or adaptation to hypoxia remains to be elucidated.

Results of previous studies[5, 7] and the present study indicate that reoxygenation causes a prostaglandin-mediated contraction. We suggest that reoxygenation after a brief period of hypoxia leads to a period of increased hydrogen peroxide generation, which appears to induce prostaglandin production, leading to the observed contraction. In addition, this response does not depend on the endothelium, because it is present in endothelium-removed vessels.

Currently we do not know what possible physiologic role these oxygen-induced mechanisms may play in the placental circulation; however, the contraction to increased Po_2 has been suggested as a contributor to closure of the umbilical circulation at birth.[4, 5] Further studies are needed to determine if the reoxygenation-elicited contraction could contribute to vasospasm associated with disease processes that affect the placental circulation.

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Inhibitory effect of medroxyprogesterone acetate on angiogenesis induced by human endometrial cancer

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OBJECTIVE: The purpose of this study was to investigate the effect of medroxyprogesterone acetate on angiogenesis induced by endometrial cancer and to elucidate the possible mechanisms by which it inhibits the growth of the cancer.

STUDY DESIGN: Tumors were obtained from 29 patients with endometrial adenocarcinoma, and angiogenesis was assayed in corneas of white rabbits.

RESULTS: Transplantation of tumor tissues into rabbit corneas induced angiogenesis in 70.4% of the corneas, but their transplantation with a pellet of medroxyprogesterone acetate induced angiogenesis in only 21.5% of the corneas. This compound also inhibited angiogenesis induced by acidic fibroblast growth factor and transforming growth factor-α.

CONCLUSION: Inhibition of angiogenesis may be one mechanism by which medroxyprogesterone acetate inhibits the growth of endometrial adenocarcinoma, and its inhibition of neovascularization induced by adenocarcinoma may be through its direct action on endothelial cells. (Am J Obstet Gynecol 1992;167:207-11.)

Key words: Medroxyprogesterone acetate, endometrial cancer, angiogenesis

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The progressive growth of tumors is associated with neovascularization induced by an angiogenic factor(s) that is secreted by the tumors.[1, 2] Therefore inhibition of neovascularization could be effective for inhibiting tumor growth.[2]

Since Anderson[3] first reported a favorable response in patients with endometrial adenocarcinoma to the potent progestin, medroxyprogesterone acetate, many patients with advanced endometrial adenocarcinoma have been treated with this compound. Clinical studies have shown its efficacy for treatment of endometrial

cancer, although one third of patients with endometrial adenocarcinoma respond objectively to progestin therapy.[4, 5] We previously found that the growth of human endometrial adenocarcinoma cells in primary culture was significantly suppressed by medroxyprogesterone acetate[6]; a growth-inhibitory effect in a nude mouse system has been also reported.[7] On the other hand, Gross et al.[8] and Oikawa et al.[9] have shown by rabbit cornea assay that medroxyprogesterone acetate inhibits angiogenesis induced by rabbit V2 carcinoma, mouse B-16 melanoma, and rat mammary tumor. Although medroxyprogesterone acetate seems to act directly on endometrial cancer to inhibit cancer growth, these findings suggest that its inhibition of angiogenesis may be one mechanism by which it inhibits the growth of endometrial adenocarcinoma. To obtain further information on its mechanism of action in human endometrial cancer, in this study we examined its effect on angiogenesis induced by adenocarcinoma.

Material and methods

Subjects. Tumor specimens were obtained at hysterectomy from 29 patients with primary endometrial adenocarcinoma who had not received previous treatment by radiotherapy, chemotherapy, or hormone therapy. The specimens obtained were immediately immersed in Eagle's minimum essential medium (Handai Biken, Osaka) containing 200 U/ml of penicillin and 200 μg/ml of streptomycin. Each specimen was divided into three equal parts, which were used for progestin receptor assay, histopathologic examination, and angiogenic assay, respectively. The specimens for receptor assay were stored at −80° C and used for assay within a week. Tumor specimens for angiogenic assay were transplanted into rabbit corneas within 3 hours. All specimens were graded histologically as previously described.[10]

Progestin receptor assay. The procedure for assay of cytoplasmic progestin receptors was as previously reported.[10] All procedures were carried out at 0° to 4° C. The tumor tissue was rapidly thawed, and tissue without blood clots or mucus was homogenized in 4 to 10 volumes of phosphate buffer (5 mmol/L sodium phosphate, 5 mmol/L mercaptoethanol, 3 mmol/L magnesium chloride, 10 mmol/L sodium molybdate, and 20% glycerol, pH 8.0). The homogenate was centrifuged in a Hitachi RPS 65 rotor (Hitachi Sales Corp., Tokyo) at 105,000*g* for 60 minutes. Duplicate samples of 0.2 ml of the supernatant (2 to 6 mg/ml of protein) were incubated for 16 hours with a saturating concentration of 10 nmol/L tritiated promegestone (3.22 TBq/mmol; New England Nuclear, Boston) and a 200-fold excess of cortisol. Nonspecific binding was determined by parallel incubations with tritiated promegestone plus cortisol in the presence of a 200-fold excess of promegestone in a final volume of 0.25 ml. The mixture was then incubated further with 25 μl of 2.5% charcoal–0.025% dextran solution for 15 minutes. The charcoal was removed by centrifugation, and the radioactivity of a 0.1 ml aliquot of the supernatant was counted. Samples with progestin receptor levels of >10 fmol/mg protein were classified as receptor positive.

Assay of angiogenesis. Bioassay of angiogenesis with rabbit cornea was carried out as described by Gimbrone et al.[11] All procedures were performed under sterile conditions. Young female Japanese white rabbits were used in the present study. A rabbit was anesthetized by injection of pentobarbital (25 mg/kg body weight) into the marginal ear vein. The eyeball was extruded forward and fixed with a rubber sheet with a hole in it smaller than the eyeball. A 2 mm incision was made in the center of the cornea, and a 2 mm corneal pocket was created by separating the lamella of the corneal stroma with an iris spatula. The base of the pocket was about 1.5 mm from the corneal limbal junction. A 1 mm^3 pellet of ethylene vinyl acetate polymer[12] with or without medroxyprogesterone acetate (200 μg) was placed at the base of the pocket, and then a 1.5 mm^3 piece of tumor was introduced with a trocar needle. Tumor fragments obtained from a patient were transplanted into six corneas of three rabbits, one eye of each rabbit with medroxyprogesterone acetate and the other without as a control. In some experiments a pellet containing 1 μg acidic fibroblast growth factor (aFGF) or 1 μg transforming growth factor-α (TGF-α) instead of a piece of tumor was transplanted into the pocket of the cornea, with or without a pellet containing medroxyprogesterone acetate (200 μg) or progesterone (200 μg).

Ten days after the transplantation, angiogenesis from the limbus was evaluated. For this the rabbit was anesthetized and the eyeball was fixed as described above. Angiogenesis from the limbus was evaluated macroscopically, and the results were confirmed by reexamining the excised corneas under a dissection microscope (Fig. 1). Corneas with inflammatory change or those that had lost tumor fragments were excluded from the evaluation. The excised corneas were fixed in 10% buffered formalin. Sections of 5 μm thickness were prepared and stained with hematoxylin and eosin. The study was carried out according to the guidelines for the care and use of the animals approved by Osaka University Medical School.

Statistics. Results were analyzed statistically by the Mantel-Haenszel test and Breslow-Day test or χ^2 test.

Results

In the control group all tumor fragments from 29 patients with endometrial adenocarcinoma induced angiogenesis in at least one of three corneas. It was therefore considered that the effect of medroxyprogesterone acetate could be evaluated on all 29 tumors. Be-

Table I. Effect of medroxyprogesterone acetate on angiogenesis induced by progestin receptor–positive and progestin receptor–negative endometrial adenocarcinomas

Progestin receptors	*Treatment*	*No. of corneal grafts*	*Corneas with angiogenesis*	
			No.	*%*
Positive (n = 17)	Control	38	24	63.2
	Medroxyprogesterone acetate	44	10	22.7*
Negative (n = 11)	Control	30	23	76.7
	Medroxyprogesterone acetate	32	5	15.6*

The Mantel-Haenszel test and the Breslow-Day test were used for statistical analysis.

*Significant difference from control, $p < 0.01$.

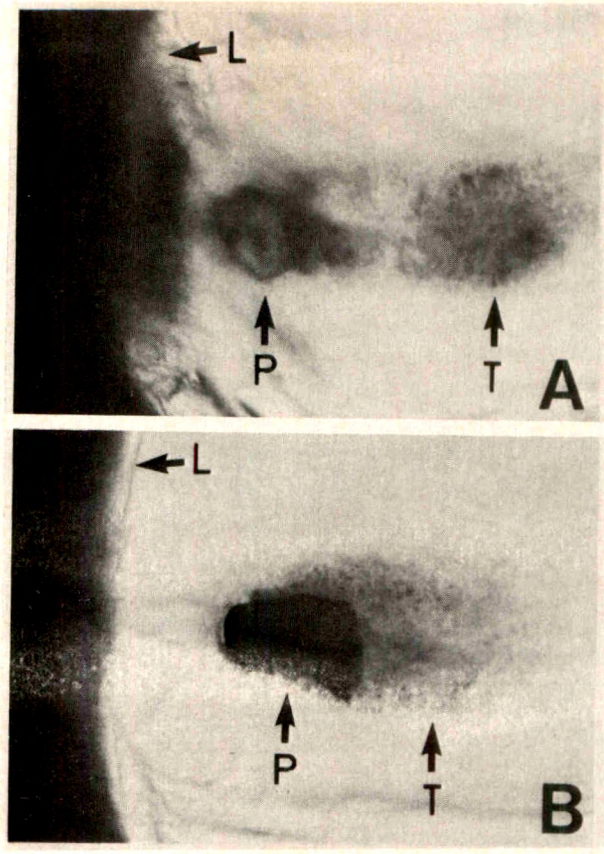


Fig. 1. Angiogenesis induced by endometrial adenocarcinoma 10 days after transplantation of tumor grafts. Eyes were examined with dissection microscope. Marked neovascularization from limbus *(L)* toward tumor graft *(T)* is seen in control eye **(A).** No angiogenesis is seen in contralateral eye in presence of medroxyprogesterone acetate **(B).** *P,* Pellet with or without medroxyprogesterone acetate in cornea.

cause corneas with inflammatory changes and those that had lost tumor fragments were omitted, the effective number of corneal grafts was 71 in the control group and 79 in the experimental group. The adenocarcinoma tissues induced angiogenesis in 50 of 71 (70.4%) control corneas but in only 17 of 79 (21.5%) corneas in the presence of medroxyprogesterone acetate (Fig. 1). The difference between these values for the control and experimental groups was statistically significant ($p < 0.01$). These results indicate that medroxyprogesterone acetate inhibited angiogenesis induced by endometrial adenocarcinoma.

We next examined whether the inhibitory action of medroxyprogesterone acetate on angiogenesis is mediated through progestin receptors in the adenocarcinomas. Of 28 adenocarcinomas examined, 17 were positive for progestin receptors. As shown in Table I, medroxyprogesterone acetate prevented induction of angiogenesis by receptor-positive and receptor-negative tumors to similar extents. In its presence angiogenesis occurred in 10 of 44 (22.7%) corneas containing receptor-positive tumors and in five of 32 (15.6%) corneas containing receptor-negative tumors. These observations suggest that the inhibitory action of medroxyprogesterone acetate on angiogenesis is not mediated through progestin receptors in adenocarcinoma.

With regard to the histologic grade of an adenocarcinoma, 22 tumors were classified as grade 1, four as grade 2, and three as grade 3. There was no apparent difference in the angiogenic activities of tumors of different grades of differentiation in the control group (Table II). In the medroxyprogesterone acetate–treated group, angiogenesis occurred in 12 of 60 (20.0%) corneas containing grade 1 adenocarcinomas and in five of 19 (26.3%) corneas containing grade 2 and 3 tumors. Thus medroxyprogesterone acetate inhibited angiogenesis regardless of the histologic grading of the adenocarcinoma.

The histologic appearance of tumor grafts 10 days after transplantation is shown in Fig. 2. In both the control (Fig. 2, *A*) and medroxyprogesterone acetate–treated (Fig. 2, *B*) group the tumor cells of grade 1 were viable, and there was no apparent difference in the histology of the grafts in the two groups.

Pellets containing the angiogenic factors, aFGF or TGF-α, without tumor tissue induced angiogenesis in all the corneas examined (Table III). Medroxyprogesterone acetate inhibited angiogenesis induced by aFGF in eight of nine corneas and prevented angiogenesis by TGF-α in all five corneas tested.

Finally we examined whether medroxyprogesterone acetate acted as progestin in inhibition of neovascularization. For this a pellet containing 200 μg of progesterone instead of the same amount of medroxyprogesterone acetate was transplanted into corneas. However, progesterone did not inhibit angiogenesis induced by aFGF in any of the nine corneas examined (Table III).

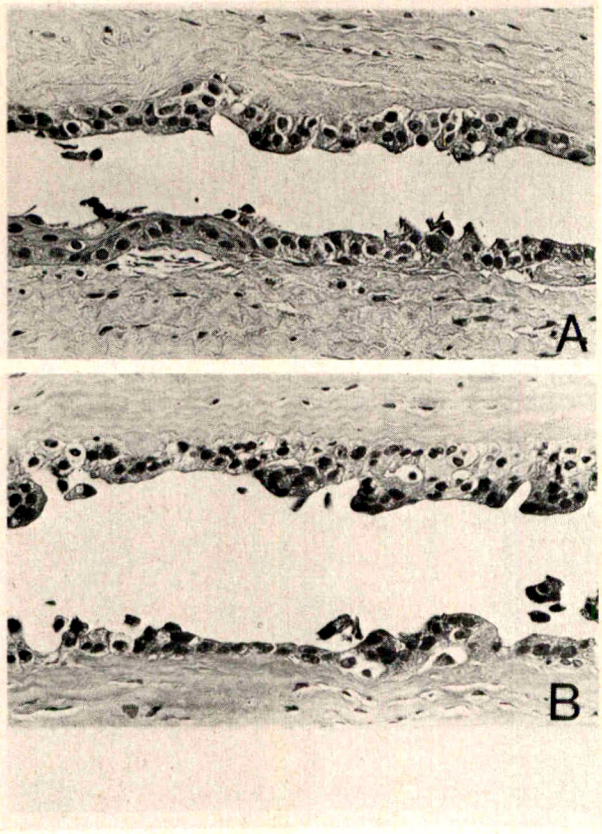


Fig. 2. Histologic appearance of grafts of endometrial adenocarcinoma of grade 1 on day 10 after transplantation. Grafts without **(A)** and with **(B)** medroxyprogesterone acetate were both viable.

Table II. Effect of medroxyprogesterone acetate on angiogenesis induced by endometrial adenocarcinomas of various histologic grades

Histologic grade	*Treatment*	*No. of corneal grafts*	*Corneas with angiogenesis*	
			No.	*%*
Grade 1 (n = 22)	Control	54	39	72.2
	Medroxyprogesterone acetate	60	12	20.2*
Grade 2 and 3 (n = 7)	Control	17	11	64.7
	Medroxyprogesterone acetate	19	5	26.3*

The Mantel-Haenszel test and the Breslow-Day tests were used for statistical analysis.

*Significant difference from control, $p < 0.01$.

Table III. Effects of medroxyprogesterone acetate and progesterone on aFGF- or TGF-α-induced angiogenesis

Angiogenic factor	*Treatment*	*No. of corneal transplants*	*Corneas with angiogenesis*	
			No.	*%*
aFGF	Control	16	16	100
	Medroxyprogesterone acetate	9	1	11.1*
	Progesterone	9	9	100
TGF-α	Control	5	5	100
	Medroxyprogesterone acetate	5	0	0*

The χ^2 test was used for the statistical analysis.

*Significant difference from control, $p < 0.01$.

Comment

To our knowledge this is the first report that medroxyprogesterone acetate inhibits angiogenesis in endometrial cancer. Angiogenesis was induced by adenocarcinoma tissues in 70.4% of the rabbit corneas tested in the absence of medroxyprogesterone acetate but in only 21.5% of those tested with medroxyprogesterone acetate. We previously showed that it inhibited the growth of cultured adenocarcinoma cells,[6] and it has been shown to decrease the circulating level of estrogen, which results in regression of adenocarcinomas.[13] The antiangiogenic effect of medroxyprogesterone acetate shown in the present study may be another mechanism by which this compound inhibits the progressive growth of adenocarcinomas.

Recently several studies have shown that human endometrial adenocarcinoma cells produce an angiogenic factor(s)[14-17] and that medroxyprogesterone acetate reduced the activity of this angiogenic factor(s) in an extract of endometrial adenocarcinoma cells in vitro.[17] These results suggest that medroxyprogesterone acetate may prevent the progressive growth of endometrial adenocarcinoma by inhibiting their angiogenic factor(s). On the other hand, in rabbit cornea medroxyprogesterone acetate inhibited angiogenesis and collagenolysis induced by an extract of a human hepatoma.[8] Moreover, it significantly suppressed the activity of a plasminogen activator produced by bovine endothelial cells in vitro.[18] These findings also suggest that medroxyprogesterone acetate may act on the host endothelial cells to inhibit angiogenesis.

We have suggested that in a cell culture system the antiproliferative effect of progestin on endometrial adenocarcinoma cells is mediated through progestin receptors in the cells.[6, 19] Clinical studies have shown that endometrial adenocarcinomas that have progestin receptors are likely to respond to progestin therapy.[4, 5] In the present study, however, medroxyprogesterone acetate inhibited angiogenesis of both progestin receptor-positive and receptor-negative tumors to similar extents, suggesting that its antiangiogenic effect was not mediated through progestin receptors in the adenocarcinomas. Medroxyprogesterone acetate almost completely inhibited the neovascularization induced by the

angiogenic factors aFGF and TFG-α, but progesterone did not influence the activities of these factors. These findings support the conclusion that the antiangiogenic action of medroxyprogesterone acetate is not mediated through progestin receptors.

A statistically significant relationship has been demonstrated between tumor grading of endometrial adenocarcinomas and their response to medroxyprogesterone acetate therapy.[4, 5] A previous in vitro study showed that its cytocidal action is more pronounced in well-differentiated adenocarcinomas.[20] In the present study, however, medroxyprogesterone acetate had antiangiogenic effects on both well-differentiated and poorly differentiated adenocarcinomas. Furthermore, microscopic examination of tumor grafts showed that adenocarcinoma cells were viable in both the presence and absence of medroxyprogesterone acetate. Therefore the antiangiogenic effect on adenocarcinomas was unlikely to be due to a cytocidal action of the compound. Previous studies have shown that an antiangiogenic effect on tumors may be inactivation of the endothelial cells that cause neovascularization.[8, 18] In the current study medroxyprogesterone acetate inhibited neovascularization induced by the angiogenic factors aFGF and TGF-α. Thus these results, together with the previous findings,[8, 18] strongly suggest that medroxyprogesterone acetate acts on endothelial cells and inhibits angiogenesis in endometrial adenocarcinoma, although the possibility that it reduces the production of an angiogenic factor(s) by adenocarcinomas cannot be ruled out.

Medroxyprogesterone acetate inhibited angiogenesis induced by both progestin receptor–positive and progestin receptor–negative adenocarcinomas, and by both well-differentiated and poorly differentiated adenocarcinomas. On the other hand, most of the adenocarcinomas that respond to therapy are progestin receptor positive and well differentiated.[4, 5] This discrepancy between clinical data on medroxyprogesterone acetate therapy and experimental data on its antiangiogenic effect suggests that the antitumor effect of medroxyprogesterone acetate on adenocarcinoma cells plays an important role in the treatment of endometrial cancer, in concert with its inhibitory action on angiogenesis.

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Changes in expression of the cyclooxygenase gene in human fetal membranes and placenta with labor

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OBJECTIVE: Our objective was to examine the expression of the gene coding for cyclooxygenase, the central enzyme in prostaglandin synthesis, in human placenta and fetal membranes during pregnancy and before and after labor at term.
STUDY DESIGN: Expression of the gene for cyclooxygenase was examined with Northern hybridization to ribonucleic acid from human placenta throughout pregnancy and human amnion and chorion decidua in the late third trimester.
RESULTS: Expression was undetectable in trophoblast during the first and second trimesters. Expression in amnion and trophoblast increased 3.5- and 2.5-fold, respectively, in association with labor.
CONCLUSIONS: Our results suggest that the increase in prostaglandin synthesis within the uterus that is seen with the onset of labor is associated with an increase in the expression of the gene cyclooxygenase.
(Am J Obstet Gynecol 1992;167:212-6.)

Key words: Gene, cyclooxygenase, labor, amnion, trophoblast

Recently, considerable interest has centered on the role of the fetal membranes in production of prostaglandins, and other eicosanoids in association with human parturition. Labor is associated with changes in the metabolism of the prostaglandin precursor arachidonic acid within the fetal membranes, particularly the amnion.[1] Before labor, archidonic acid metabolism in amnion is primarily through the lipoxygenase enzymes. There is little or no metabolism by cyclooxygenase enzyme pathways to prostaglandins.[2, 3] In association with labor overall arachidonic acid metabolism in amnion increases. The majority of this increase is in cyclooxygenase metabolism to prostaglandin E_2 (PGE_2). Human chorion contains large concentrations of prostaglandin dehydrogenase and is principally a site of prostaglandin inactivation.[4] Human placenta produces both cyclooxygenase and lipoxygenase metabolites of arachidonic acid and an epoxygenase product.[3, 5] Both in the first trimester and after spontaneous labor, placenta has high arachidonic acid metabolic activity, producing cyclooxygenase but principally lipoxygenase and epoxygenase products. At term, before labor, its metabolic activity is significantly reduced.[5]

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A number of substances are known to stimulate prostaglandin metabolism by the fetal membranes and are considered as candidates for the control of human parturition. Platelet-activating factor stimulates prostaglandin production in amnion, probably by increasing intracellular Ca^{2+} concentrations and thereby increasing phospholipase activity. Platelet-activating factor is present in amniotic fluid during labor but not before labor. Because platelet-activating factor may be secreted by the fetal lungs, a link has been postulated between fetal lung maturation and initiation of labor.[6] Interleukins (IL) have also been postulated to be the signal for the onset of human parturition.[7] Human decidua is a source of IL-1β. IL-1β stimulates prostaglandin synthesis by amnion and decidua. There appears to be an increase in IL-1β activity in amniotic fluid in the third trimester and a further increase in association with parturition. IL-1β has been shown to stimulate prostacyclin synthesis in human umbilical vein endothelial cells by stimulating an increase in the expression of the gene coding for cyclooxygenase.[8]

It has been postulated that the increase in prostaglandin synthesis seen in the fetal membranes in association with labor is due to an increase in phospholipase activity and a consequent increase in availability of arachidonic acid. However, the accompanying change in the cyclooxygenase lipoxygenase metabolism ratio suggests that there must also be an increase in the activity of the cyclooxygenase enzyme. The complementary deoxyribonucleic acid (cDNA) sequence of cyclooxygenase has been characterized in sheep, mouse, and human.[9-11] Unfortunately, the human cDNA clone has not been made available to us. The sheep cycloox-

ygenase enzyme shows a high degree of homology to the human.[12] We have previously demonstrated that cyclooxygenase is a highly conserved gene found in a wide range of species and that the sheep cDNA may be used to detect expression of the human cyclooxygenase gene by Northern blotting.[12]

To identify whether labor is associated with increased expression of the cyclooxygenase gene we have performed experiments to examine the in vivo expression of cyclooxygenase in human placenta throughout pregnancy and in human amnion and chorion at term before and after spontaneous labor.

Methods

Tissues. Placental tissue was collected at termination of pregnancy by dilatation and suction evacuation at 5, 8, 10, and 12 weeks of amenorrhea and by dilatation and instrumental evacuation at 14 and 20 weeks. Placental tissue was identified macroscopically, and the tissue type was confirmed histologically. Fetal membranes and placenta were collected after delivery by cesarean section at 30 weeks for essential hypertension (one case) and at term, before the onset of labor, for previous cesarean section in otherwise uncomplicated pregnancies (10 cases). Fetal membranes and placenta were also collected from 10 cases of spontaneous labor and delivery at term in uncomplicated pregnancies. Labor was complete in each case within 10 hours. There was no evidence of infection in any of the cases studied.

Ribonucleic acid (RNA) isolation and Northern hybridization. To limit degradation, RNA was prepared from each of the samples immediately after collection. The amnion was carefully peeled from the chorion. The maternal surface of the term placenta was cut away and a tissue sample was taken from beneath it. All three tissues were washed thoroughly in phosphate-buffered saline solution to remove all traces of blood and were then snap-frozen dry in liquid nitrogen. Total RNA was isolated from each tissue with a guanidinium-phenol-chloroform method.[13] The yield of RNA was determined spectrophotometrically. Thirty micrograms of total RNA was denatured at 60° C in 75% formamide and 24% formaldehyde for 2 minutes. It was then loaded onto a 1.5% agarose gel containing 3% formaldehyde and then separated by electrophoresis. The RNA within the gel was denatured in 0.05 mol/L sodium hydroxide for 20 minutes and then transferred by Northern blot onto Hybond nylon filters (Amersham). Blots were baked at 80° C for 2 hours and prehybridized in 50% formamide, 0.75 mol/L sodium chloride, 0.075 mol/L sodium citrate, 50 mmol/L sodium phosphate buffer (pH 6), 250 μg/ml denatured salmon sperm DNA, and 10× Denhardt's solution at 42° C overnight.

A 1.8 kb *Eco*RI fragment of the sheep cyclooxygenase cDNA,[9] subcloned in our laboratory, was radiolabeled by the method of Feiberg and Vogelstein.[14] Hybridization to the Northern blots was performed overnight at 42° C in the same solution as that used for prehybridization. Filters were washed sequentially in 5×, 2×, 1×, and 0.5× saline solution–sodium citrate buffer. Autoradiography was undertaken for 5 days at −70° C with intensifying screens.

After autoradiography the cyclooxygenase cDNA probe was stripped from the filters. Filters were placed in double-distilled water at 98° C, and the water was allowed to cool slowly at room temperature. The filters were then prehybridized and hybridized to a radiolabeled cDNA for human β-actin with the same protocols. Filters were washed sequentially in 2×, 1×, 0.5×, and 0.1× saline solution–sodium citrate buffer. Autoradiography was undertaken for 18 hours at −70° C with intensifying screens.

Expression of cyclooxygenase and β-actin was assessed densitometrically. β-Actin expression was used to confirm uniformity of loading. To compensate for nonuniform RNA loading, the density of signal as a result of hybridization of the cyclooxygenase probe to the Northern blot is expressed as a ratio of the β-actin signal density. Statistical analysis was performed with the Student *t* test.

Results

RNA of good quality was isolated from each of the tissues. β-Actin was expressed in all of the tissues at all gestational ages.

Differences in density of the β-actin transcript band were seen where RNA loading was not uniform; otherwise there were no apparent differences in the expression of β-actin. Expression of cyclooxygenase was not detectable in trophoblast at 5, 8, 10, 12, 14, 20, or 30 weeks but was seen on positive controls on the same filter. At term before labor, cyclooxygenase expression was seen at very low levels in amnion in four cases, chorion in two cases, and trophoblast in four cases. After the spontaneous onset of labor, expression was seen in all the amnion and placental samples and in six of the 10 chorion-decidual samples. Overall there was a 3.5-fold increase in the expression of cyclooxygenase in amnion($p < 0.005$) and a 2.5-fold increase in its expression in trophoblast ($p < 0.005$) (Figs. 1 and 2). The small increase seen in expression in chorion-decidua did not reach statistical significance.

Comment

We have demonstrated that expression of cyclooxygenase is not detectable by Northern analysis in human trophoblast in the first or second trimester of pregnancy. At term, before labor, cyclooxyenase is expressed at low levels in amnion, chorion-decidua, and

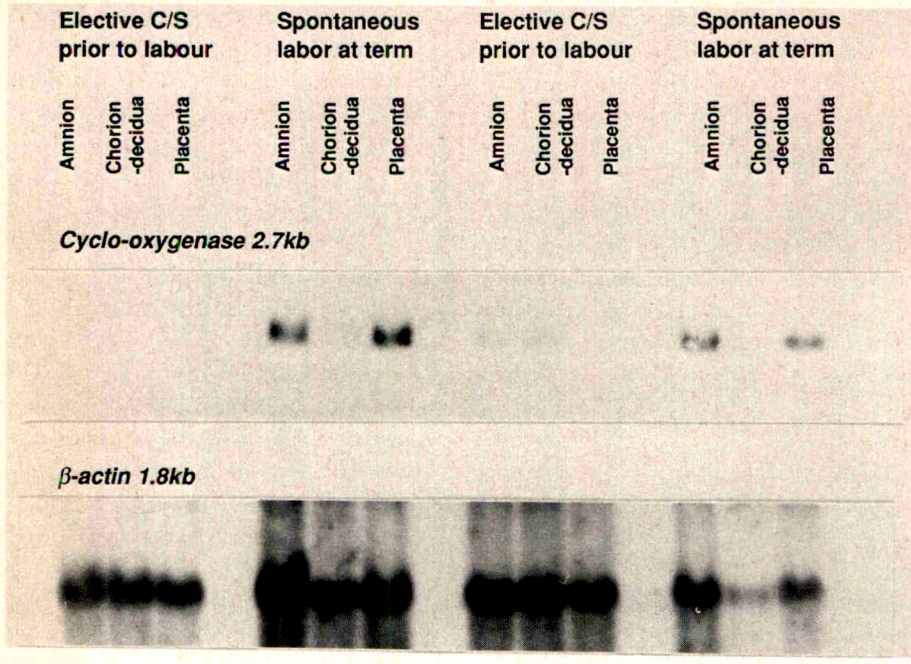


Fig. 1. Northern blot showing cyclooxygenase expression in amnion, chorion-decidua, and placenta collected at elective cesarean section *(C/S)* before onset of labor or after spontaneous onset of labor. Expression of β-actin is shown to control for uneven RNA loading.

trophoblast. In association with labor there is a large increase in expression in amnion and trophoblast. These findings are in accord with previous studies that have shown that arachidonic acid metabolism in amnion is essentially by lipoxygenase metabolic pathways until the onset of parturition, when there is a large increase in cyclooxygenase metabolism to prostaglandins.[2] It has been shown that bacterial products and inflammatory cytokines may stimulate PGE_2 production by fetal membranes.[2, 7] It is possible that the increase in cyclooxygenase expression seen in the fetal membranes after spontaneous delivery may be a response to bacterial exposure. There was, however, no evidence of either intrapartum or puerpural sepsis in any of the patients studied nor were there any associated neonatal infections.

Chorion is principally a site of prostaglandin catabolism. The cyclooxygenase expression seen in chorion-decidua is probably mostly within the decidual part of this tissue. Decidua is composed of lymphocytes, which represent 20% of cell types at term, and decidual stromal cells. An increase in decidual prostaglandin synthesis with labor has been demonstrated,[1] and it has been shown that there is an increase in cyclooxygenase enzyme levels in decidual stromal cells with labor.[15] Although we have seen a small increase in cyclooxygenase expression with labor in chorion-decidua, this did not reach statistical significance. It is probable that any increase in decidual stromal cyclooxygenase expression has been masked in our experiments by the presence of RNA from chorion and nonstromal decidual cells. This could be confirmed by in situ hybridization experiments.

We have failed to demonstrate cyclooxygenase expression in placenta during the first trimester. First-trimester placenta has been shown to produce cyclooxygenase products.[5] Therefore, there must be some expression of cyclooxygenase in first trimester trophoblast but at a level below the sensitivity of our analysis. Because we did not separate placental trophoblast from connective tissue, it is possible that the presence of non-trophoblast RNA may be diluting the trophoblast RNA and reducing the apparent expression of cyclooxygenase. Maier et al.[8] have demonstrated that unstimulated human umbilical vein endothelial cells express the cyclooxygenase messenger RNA (mRNA) at levels undetectable by Northern hybridization. Our results suggest that this may also be the case in human trophoblast in the first and second trimesters of pregnancy. It is possible that either cyclooxygenase mRNA or the protein itself has increased stability in the first trimester. We have found a large increase in cyclooxygenase expression in placenta in association with labor. This is consistant with the increase in synthesis of cyclooxygenase products seen in trophoblast with parturition.[5]

The synthesis of prostaglandins in any tissue follows well-established pathways. The substrate arachidonic acid is released from intracellular phospholipids by the

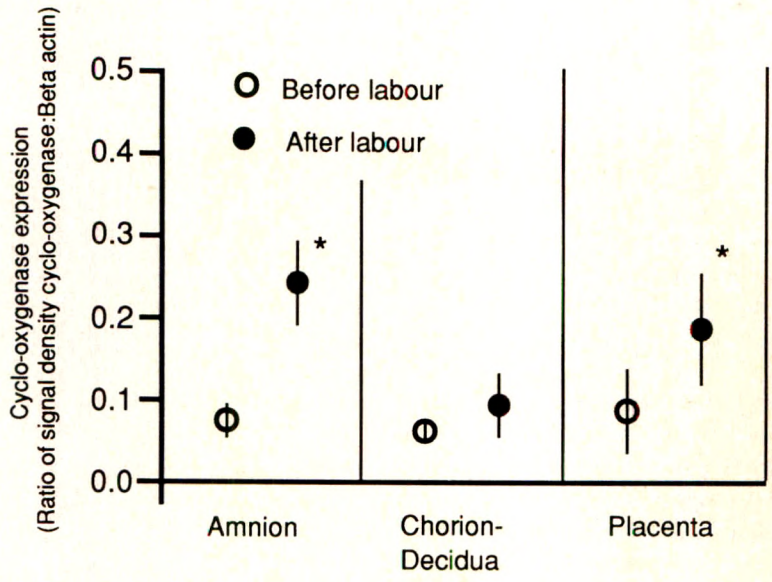


Fig. 2. Cyclooxygenase expression as ratio of signal density cyclooxygenase to that of β-actin in amnion, chorion-decidua, and placenta collected at elective cesarean section before onset of labor or after spontaneous onset of labor. Mean ± SD, $n = 10$. *Asterisk*, $p < 0.05$.

action of phospholipases. In a reaction catalyzed by cyclooxygenase, free arachidonic acid is converted to unstable prostaglandin endoperoxides. These prostaglandin endoperoxides are then converted by specific synthases to whichever prostaglandin, prostacyclin, or thromboxane is produced by the specific tissue. Recent theories of the initiation of human parturition have concentrated on phospholipase activity as the rate-limiting step in the synthesis of prostaglandins. Cyclooxygenase activity has not been considered to be rate limiting because an immediate rise in prostaglandin synthesis can be seen after addition of exogenous arachidonic acid to intrauterine tissues. However, cyclooxygenase has a very short life and undergoes a "suicide reaction" by inactivation of the apoprotein moiety of the enzyme by free radical species generated during catalysis.[16] It is highly unlikely that cyclooxygenase is simply waiting within the cell for an increase in phospholipase activity to increase its substrate availability. It is more likely that the increase in total arachidonic acid synthesis and in cyclooxygenase metabolism to PGs is caused by an increase in phospholipase activity coupled with increased synthesis of the cyclooxygenase enzyme. Our data support this theory.

There is some controversy as to which tissue within the uterus is the prime mover in the initiation of human parturition. Amniotic epithelium is a popular candidate because it contains the highest concentration of arachidonic acid of all tissues within the uterus[17] and demonstrates a significant increase in prostaglandin synthesis in association with labor.[1, 2] The chorion is principally a site of prostaglandin inactivation and does not itself synthesize prostaglandins. Several studies have investigated the intact transfer of prostaglandins from the amnion, across chorion-decidua, with conflicting results.[18-21] Because amnion is avascular and has no exogenous supply of precursor arachidonic acid, its increase in prostaglandin synthesis must involve an increase in the activity of both phospholipase and cyclooxygenase. We have demonstrated that the increased cyclooxygenase activity is associated with increased gene expression and therefore presumably increased enzyme synthesis. Whether this increase in cyclooxygenase expression is due solely to increased substrate availability or stimulated by other factors remains to be demonstrated. It does appear that IL-1β may directly stimulate increased expression of cyclooxygenase,[8] and this may be part of the mechanism by which this cytokine may initiate labor.

Decidua is a vascular tissue and may have a continuous exogenous supply of arachidonic acid. Control of prostaglandin synthesis in decidua is likely to be through the activity of the cyclooxygenase enzyme and, therefore, because it has a very short half-life, through the expression of the cyclooxygenase gene. Examination of the expression of cyclooxygenase in decidua will require either cell separation techniques or in situ hybridization.

Placenta is not thought to play a central role in the prostaglandin synthesis of early parturition. Nevertheless, its increase in cyclooxygenase activity after labor mirrors its overall increase in the metabolism of arachidonic acid. Like decidua, placenta has an available extracellular source of arachidonic acid. Experiments

by Rose et al.[5] have demonstrated that placenta will only synthesize cyclooxygenase products from exogenous arachidonic acid. Metabolism of endogenous substrate is only by lipoxygenase and epoxygenase pathways. Furthermore, addition of exogenous arachidonic acid to placental cells obtained before labor does not yield cyclooxygenase products. This suggests that cyclooxygenase expression in trophoblast is not controlled simply by the availability of substrate and may represent an important factor in the control of prostaglandin synthesis in this tissue.

Whichever tissue is of greatest importance in producing the prostaglandins that initiate human labor, it appears that the increase in prostaglandin synthesis must be a consequence of both substrate availability and cyclooxygenase expression. In amnion, which is avascular, this must mean an increase in mobilization of stored arachidonic acid together with an increase in cyclooxygenase expression. In tissues with a good vascular supply and therefore an exogenous supply of archidonic acid, decidua, and placenta, it is likely that prostaglandin synthesis is controlled principally by cyclooxygenase expression. An understanding of the factors that control the expression of cyclooxygenase will provide valuable information about the mechanisms of parturition in humans.

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A novel change in cytologic localization of human chorionic gonadotropin and human placental lactogen in first-trimester placenta in the course of gestation

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Cytologic localization of human chorionic gonadotropin and human placental lactogen in developing human early placenta was analyzed by avidin-biotin immunoperoxidase techniques with an affinity-purified polyclonal antibody to β-human chorionic gonadotropin carboxyl terminal peptide and a polyclonal antibody to human placental lactogen. In 4- to 5-week placentas human chorionic gonadotropin and human placental lactogen were found to be primarily localized to cytotrophoblasts, whereas in 6- to 12-week placentas these substances were exclusively localized to syncytiotrophoblast. We previously reported that a similar change in cytologic localization of epidermal growth factor and its receptor from cytotrophoblasts to syncytiotrophoblast in first-trimester placenta appeared between 5 and 6 weeks of gestation. Because epidermal growth factor was demonstrated to stimulate human chorionic gonadotropin and human placental lactogen production by early placental tissues, their simultaneous expression, as well as epidermal growth factor and its receptor in the cytotrophoblast of 4- to 5-week placenta and in the syncytiotrophoblast of 6- to 12-week placenta, implies that human chorionic gonadotropin and human placental lactogen production by first-trimester placenta may be regulated in an autocrine manner, wherein epidermal growth factor may serve as the signal. These findings suggest that in very early placenta, before 6 weeks of gestation, no sequential expression of human chorionic gonadotropin and human placental lactogen closely linked to syncytia formation may exist and that both can be expressed in the cytotrophoblast or undifferentiated stem cell of villous trophoblast in very early placenta. (Am J Obstet Gynecol 1992;167:217-22.)

Key words: Placenta, cytotrophoblast, human chorionic gonadotropin, human placental lactogen, immunohistochemistry

The villous trophoblast of human placenta is composed of two distinct layers: an inner cell layer of mononuclear cytotrophoblast and an outer cell layer of multinuclear syncytiotrophoblast. It is generally accepted that the cytotrophoblast is the undifferentiated stem cell of the villous trophoblast and that the syncytiotrophoblast is the fully differentiated, end-stage cell derived from cytotrophoblast.[1-6]

The human placental trophoblast elaborates at least two peptide hormones, human chorionic gonadotropin (hCG) and human placental lactogen (hPL). A number of immunohistochemical studies,[7-10] including ours,[11] have indicated that immunoreactive β-hCG, hCG, and hPL are exclusively localized to the syncytiotrophoblast, whereas other immunohistochemical studies[12, 13] have suggested that the cytotrophoblast may contain immunoreactive α-hCG. Consistent with the immunohistochemical findings, Hoshina et al.,[14, 15] using in situ hybridization techniques to localize hCG (α and β) and hPL messenger ribonucleic acids in placental tissue sections, showed that the expression of α-hCG messenger ribonucleic acid is initiated in some differentiating cytotrophoblasts, whereas the expression of β-hCG messenger ribonucleic acid starts during the process of differentiation of syncytial trophoblast and hPL messenger ribonucleic acid is expressed only in the fully differentiated syncytiotrophoblast. However, it must be pointed out that the placental tissue materials used in the studies reported have been obtained at a gestational age later than 6 weeks, probably because of the greater availability of placental materials between 6 and 12 weeks of gestation and at term. Thus the claim that the ability of human trophoblast to synthesize hCG and hPL is tied to syncytia formation may be limited to the

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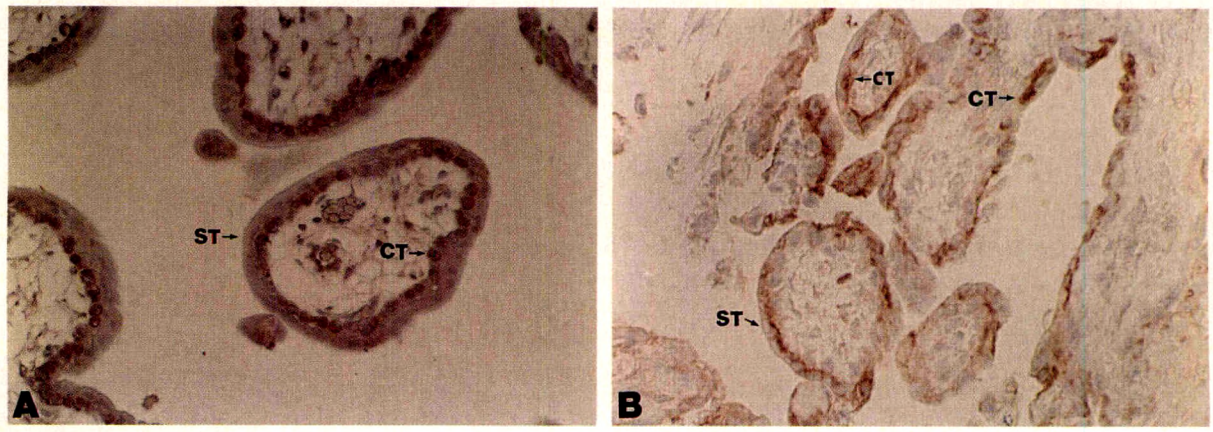


Fig. 1. Immunohistochemical localization of β-hCG carboxyl terminal peptide *(hCGβ-CTP)* and hPL in very early first-trimester placenta. Formalin-fixed, paraffin-embedded sections of 4-week placenta were stained with anti-β-hCG-CTP antibody **(A)** and with anti-hPL antibody **(B)**. Cytotrophoblastic cells *(CT)* showed pronounced staining with anti-β-hCG-CTP antibody and with anti-hPL antibody, whereas syncytiotrophoblast *(ST)* had scarce staining. (Original magnification ×200.)

placenta with a gestational age >6 weeks. Nevertheless, it is known that hPL exerts a lipolytic and glucose-sparing effect on the mother to ensure that the nutritional demands of the fetus are met,[16, 17] whereas hCG is responsible for the rescue of the corpus luteum, which is indispensable for the maintenance of very early pregnancy during the first 4 to 6 weeks of gestation.[18] Thus it is of great importance to provide insight into the expression of hCG and hPL in very early placenta before 6 weeks of gestation.

On the other hand, epidermal growth factor (EGF) has been demonstrated to stimulate the production of hCG and hPL by early placental tissues through the binding to EGF receptor.[19] Furthermore, we have recently found that EGF and EGF receptor are initially expressed in the cytotrophoblasts in very early placenta before 6 weeks of gestation and thereafter expressed in the syncytiotrophoblast of 6- to 12-week placenta.[20] A remarkable change in cytologic localization of EGF and EGF receptor in very early placenta appeared between 5 and 6 weeks of gestation. Thus it seems interesting to investigate whether cytologic localization of hCG and hPL in very early placenta may also change during the course of early gestation in association with the shift of cytologic localization of EGF and EGF receptor in first-trimester placenta. Hence in the current study attention was focused on the expression of hCG and hPL in very early placenta, before 6 weeks of gestation, and the possible change in cytologic localization of hCG and hPL in first-trimester placenta over the course of early gestation was examined.

Material and methods

Material. First-trimester placentas were obtained from 12 patients who underwent elective abortion at 4 to 5 weeks (three cases), 6 to 7 weeks (three cases), and 8 to 12 weeks (six cases) of gestation. Third-trimester placentas were obtained from four patients who had cesarean section between 38 and 40 weeks of gestation. The gestational age of the placenta was determined by the known date of conception or estimation from the date of the patient's last menstrual period. In all cases gestational age was further evaluated by ultrasonographic examination. Informed consent for the use of placental tissues for immunohistochemical studies was obtained from the patients before operation.

Immunohistochemical staining. Placental tissues obtained were fixed in 4% buffered neutral formalin, dehydrated, and embedded in paraffin. Sections, 5 to 6 μm in thickness, were deparaffinized and followed by standard histologic techniques.

Immunohistochemical staining was performed by avidin/biotin immunoperoxide techniques with a polyvalent immunoperoxidase kit (Omnitags, Lipshaw, Mich.) as previously described by Maruo and Mochizuki.[11] An affinity-purified sheep polyclonal antibody against β-hCG carboxyl terminal peptide (Takeda Chemical Industries, Osaka) and a rabbit polyclonal antibody against hPL (Dako Corp., Santa Barbara, Calif.) were used, respectively, as the primary antibodies in this study. The anti-β-hCGβ carboxyl terminal peptide antibody and anti-hPL antibody were diluted 1:100 and 1:1000 before use, respectively.

To assure specificity of the immunologic reactions, adjacent control sections were subjected to the same immunoperoxidase method, except that the primary antibodies to β-hCG carboxyl terminal peptide or hPL were respectively replaced by nonimmune sheep immunoglobulin G (Miles, Elkhart, Ind.) or nonimmune rabbit immunoglobulin G (Miles). In the controls no positive staining was observed.

Results

Fig. 1, *A* and *B*, shows the immunohistochemical staining for β-hCG carboxyl terminal peptide and hPL, respectively, in very early placenta obtained at 4 weeks

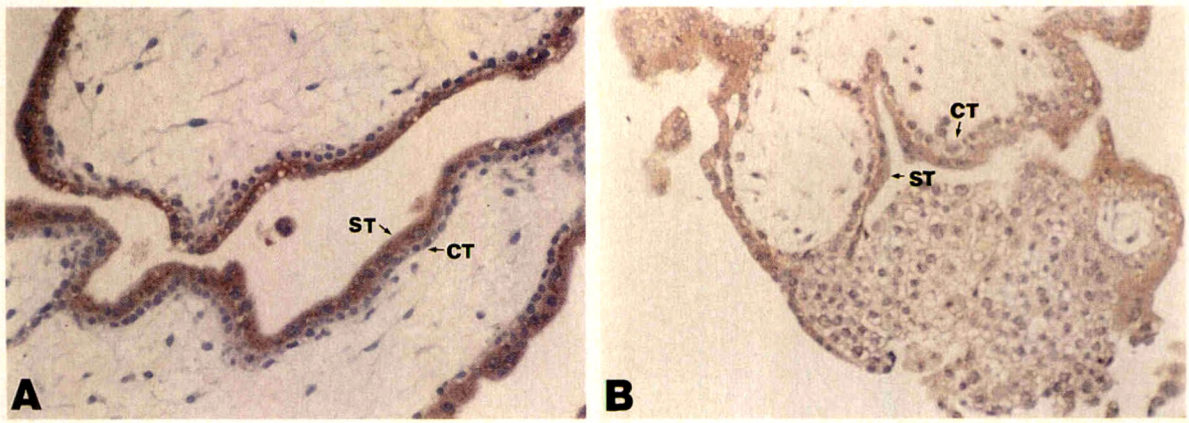


Fig. 2. Immunohistochemical localization of β-hCG-CTP and hPL in early first trimester placenta. Formalin-fixed, paraffin-embedded sections of 6-week placenta were stained with anti-β-CTP antibody **(A)** and anti-hPL antibody **(B)**. Pronounced staining for β-hCG-CTP and hPL was observed in syncytiotrophoblast *(ST)*, whereas cytotrophoblastic cells *(CT)* were negative for appreciable staining. (Original magnification ×200.)

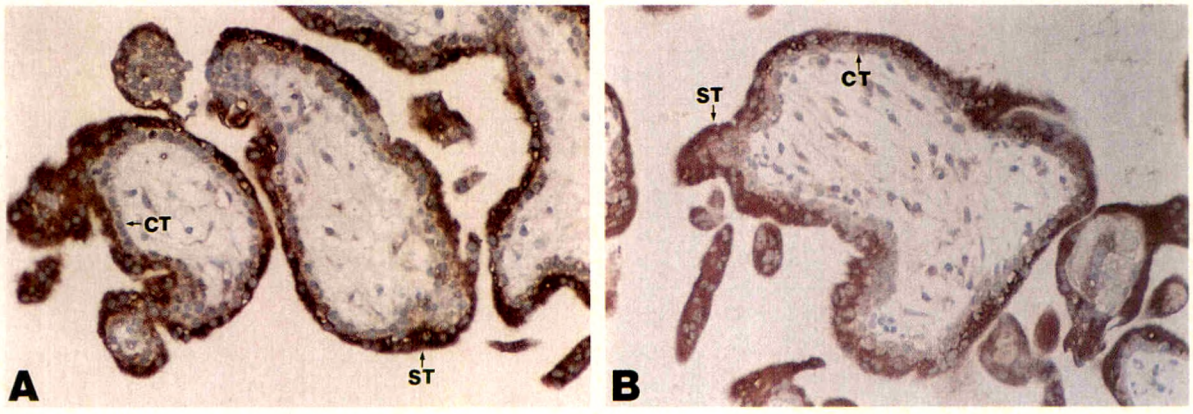


Fig. 3. Immunohistochemical localization of β-hCG-CTP and hPL in first trimester placenta. Formalin-fixed, paraffin-embedded sections of 9-week placenta were stained with anti-β-hCG-CTP antibody **(A)** and anti-hPL antibody **(B)**. Prominent staining for β-hCG-CTP and hPL was observed with maximal intensity in syncytiotrophoblast *(ST)*, whereas cytotrophoblastic cells *(CT)* were negative for appreciable staining. (Original magnification ×200.)

of gestation. Both immunostaining with anti-β-hCG carboxyl terminal peptide and anti-hPL antibody were prominent in cytotrophoblasts, whereas syncytiotrophoblast showed scarce staining for β-hCG carboxyl terminal peptide and hPL. A similar pattern of immunostaining for β-hCG carboxyl terminal peptide and hPL was observed in tissue sections of 5-week placenta.

Fig. 2, *A* and *B*, represents the immunohistochemical staining for β-hCG carboxyl terminal peptide and hPL, respectively, in early placenta obtained at 6 weeks of gestation. Unlike the very early placenta obtained at 4 to 5 weeks of gestation, both immunostaining with anti-β-hCG carboxyl terminal peptide antibody and anti-hPL antibody were exclusively localized to syncytiotrophoblast. No appreciable staining for β-hCG carboxyl terminal peptide and hPL was found in cytotrophoblasts. In the case of 8- to 12-week placenta (Fig. 3, *A* and *B*), the pattern of cytologic localization of β-hCG carboxyl terminal peptide and hPL was similar to that in 6-week placenta, but the staining intensity for β-hCG carboxyl terminal peptide and hPL of the syncytiotrophoblast was more prominent compared with that of 6-week placenta.

Fig. 4, *A* and *B*, shows the immunohistochemical staining for β-hCG carboxyl terminal peptide and hPL, respectively, in term placenta obtained at 40 weeks of gestation. Both immunostaining with anti-β-hCG carboxyl terminal peptide antibody and anti-hPL antibody were focally observed with the exclusive localization in syncytiotrophoblast.

The results of cytologic localization of β-hCG carboxyl terminal peptide and hPL in first-trimester placenta according to the type of villous trophoblast and the gestational age are summarized in Table I. A remarkable shift in cytologic localization of β-hCG carboxyl terminal peptide and hPL from cytotrophoblast

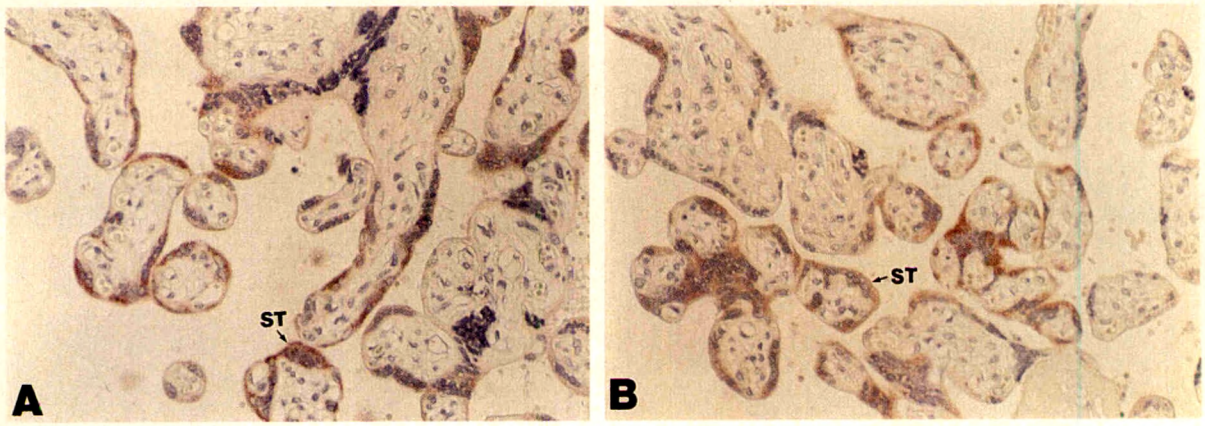


Fig. 4. Immunohistochemical localization of β-hCG-CTP and hPL in term placenta. Formalin-fixed, paraffin-embedded sections of 40-week placenta were stained with anti-β-hCG-CTP antibody **(A)** and anti-hPL antibody **(B)**. Pronounced staining for β-hCG-CTP and hPL was focally observed with exclusive localization in syncytiotrophoblast *(ST)*. (Original magnification ×200.)

Table I. Cytologic localization of β-hCG-CTP and hPL in first-trimester and term placenta according to the gestational age

	First-trimester						*Term (40 wk)*	
	4 to 5 wk		*6 to 7 wk*		*8 to 12 wk*			
	β-hCG carboxyl terminal peptide	*hPL*	*β-hCG carboxyl terminal peptide*	*hPL*	*β-hCG carboxyl terminal peptide*	*hPL*	*β-hCG carboxyl terminal peptide*	*hPL*
Cytotrophoblast	+	+	−	−	−	−	−	−
Syncytiotrophoblast	−	−	++	++	+++	+++	+	+

Semiquantitative scoring denotes intensity of immunostaining.

to syncytiotrophoblast was found to appear between 5 and 6 weeks of gestation.

Comment

The current study demonstrated that cytologic localization of hCG and hPL in human first trimester placenta changes in the advancing course of gestation. In the very early placenta obtained at 4 to 5 weeks of gestation, hCG and hPL were primarily localized to cytotrophoblasts, whereas in early placenta obtained later than 6 weeks of gestation they were exclusively localized to syncytiotrophoblast. These findings suggest that both are initially expressed in cytotrophoblasts in very early placenta before 6 weeks of gestation and thereafter expressed in syncytiotrophoblast in the placenta during the remainder of gestation. To our knowledge, the current study is the first to demonstrate the change in cytologic localization of hCG and hPL in developing human placenta in the course of early gestation.

The change in cytologic localization of hCG and hPL from cytotrophoblasts to syncytiotrophoblast in very early placenta is of great interest considering the established finding that syncytiotrophoblast is formed from cytotrophoblasts by a process of proliferation followed by cell fusion.[4, 5] In situ hybridization studies[14, 15] with α-hCG, β-hCG, and hPL probes on tissue sections of the placenta obtained later than 6 weeks of gestation have demonstrated that the villous trophoblast acquires the ability to elaborate these substances in association with the morphologic transformation from cytotrophoblasts to syncytiotrophoblast and that the biochemical differentiation of the villous trophoblast occurs only after syncytia formation. Thus it seems evident that in the placenta with gestational age later than 6 weeks there is a sequential expression of α-hCG, β-hCG, and hPL closely correlated with the differentiation of the villous trophoblast. However, when Kao et al.[21] grew purified human placental cytotrophoblasts under serum-free conditions to elucidate whether syncytia formation is a prerequisite for the biochemical differentiation of the villous trophoblast, they found that the formation of syncytiotrophoblast is not a prerequisite for the acquisition of the ability to elaborate hCG but is only one of the consequences of the differentiation program of the trophoblast. They demon-

strated that purified human placental cytotrophoblasts in culture could secrete hCG at the same rate under serum-free conditions whether they were plated on plastic only, which prevented syncytia formation, or fibronectin laminin or type IV collagen, which allowed syncytia formation to occur. Our results obtained with the tissue sections of very early human placenta support the notion that syncytia formation is not a prerequisite for the acquisition of a specific endocrine function of the placenta. In the case of very early placenta before 6 weeks of gestation, both hCG and hPL can be expressed in cytotrophoblasts.

In this regard, our recent findings in an earlier study are of considerable interest. We have demonstrated that cytologic localization of EGF and EGF receptor in developing human placenta varies over the course of gestation and that even in first-trimester placenta there is a remarkable change in the cytologic localization from cytotrophoblasts to syncytiotrophoblast at the sixth week of gestation.[20] Thus the change in cytologic localization of hCG and hPL in very early placenta observed in the course of early gestation is closely coincident with the change in cytolocalization of EGF and EGF receptor. Because EGF has been demonstrated to exert a stimulatory effect on hCG and hPL production by early placental tissues through the binding to EGF receptor,[19] the simultaneous expression of EGF and EGF receptor and hCG and hPL in the cytotrophoblast of 4- to 5-week placentas and in the syncytiotrophoblast of 6- to 12-week placentas implies that the production of hCG and hPL by first-trimester placenta may be regulated by an autocrine system, wherein EGF may serve as the signal.

It is evident that increasing amounts of hCG produced by the chorion around the time of implantation play an important role in rescuing the corpus luteum. The placenta is known to take over the corpus luteum function at the sixth week of gestation in producing progesterone and estrogen necessary for the maintenance of early gestation. It is of interest that the dynamic change in cytologic localization of hCG and hPL and EGF and EGF receptor from cytotrophoblast to syncytiotrophoblast in very early placenta also occurs at the sixth week of gestation. Taking these findings into account, the sixth week of gestation seems to be critical in the functional development of the early placenta.

These findings suggest that in very early placenta, before the sixth week of gestation, hCG and hPL can be expressed in the cytotrophoblast (the undifferentiated stem cell of the villous trophoblast) and that there is a shift in cytologic localization of hCG and hPL from cytotrophoblast to syncytiotrophoblast around the sixth week of gestation. It is therefore likely that the program of the sequential expression of the genes coding for hCG (α and β) and hPL correlated with the differentiation of the villous trophoblast may be established in the placenta at the sixth week of gestation. Before that time no sequential expression of hCG and hPL correlated with trophoblast differentiation may exist in the placenta. Further studies with in situ hybridization with hCG (α and β) and hPL probes on very early placental tissues will be needed to reinforce this notion.

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Transforming growth factor-β opposes the stimulatory effects of interleukin-1 and tumor necrosis factor on amnion cell prostaglandin E_2 production: Implication for preterm labor

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OBJECTIVE: In preterm labor increased concentrations of interleukin-1 and tumor necrosis factor are present in amniotic fluid. These cytokines may promote labor by stimulating the production of prostaglandins by intrauterine tissues. In many biologic processes, transforming growth factor-β modifies the actions of cytokines. We studied the effect of transforming growth factor-β on the cytokine-induced prostaglandin E_2 production by amnion cells.

STUDY DESIGN: Human amnion cells in monolayer culture were treated with interleukin-1, tumor necrosis factor, or vehicle in the presence or absence of transforming growth factor-β. The prostaglandin E_2 production was measured.

RESULTS: Transforming growth factor-β decreased the interleukin-1- or tumor necrosis factor–induced prostaglandin E_2 production by 70% to 80% and the basal prostaglandin E_2 synthesis by 27%. The synergistic stimulation of prostaglandin E_2 production by the combination of interleukin-1 with tumor necrosis factor was inhibited by 80% in cells treated with transforming growth factor-β. Transforming growth factor-β1, -β2, and -β1,2 were equipotent.

CONCLUSION: Transforming growth factor-β suppresses the cytokine-induced prostaglandin E_2 production by amnion cells and may be an important factor in maintaining pregnancy in the face of labor-promoting cytokines. (Am J Obstet Gynecol 1992;167:222-6.)

Key words: Preterm labor, prostaglandins, interleukin-1, tumor necrosis factor, transforming growth factor-β, fetal membranes

Preterm labor often occurs in the setting of intrauterine infection.[1] The concentrations of cytokines such as interleukin-1 (IL-1) and tumor necrosis factor (TNF) increase in amniotic fluid in preterm labor.[2-4] Prostaglandins, major inducers of uterine contractions, are produced by intrauterine tissues in response to these cytokines.[3, 5-7] The amnion, which produces almost exclusively prostaglandin E_2 (PGE_2),[8] is an important intrauterine source of prostaglandin. The combination of IL-1 with TNF has a synergistic stimulator effect on the PGE_2 production by human amnion cells in culture.[9] IL-1 and TNF have been implicated in the induction of preterm labor associated with infections.[2, 3, 10]

Transforming growth factor-β (TGF-β) represents a family of multifunctional factors that influence cell growth, differentiation, immunity, and extracellular matrix formation.[11] Several forms of TGF-β and of

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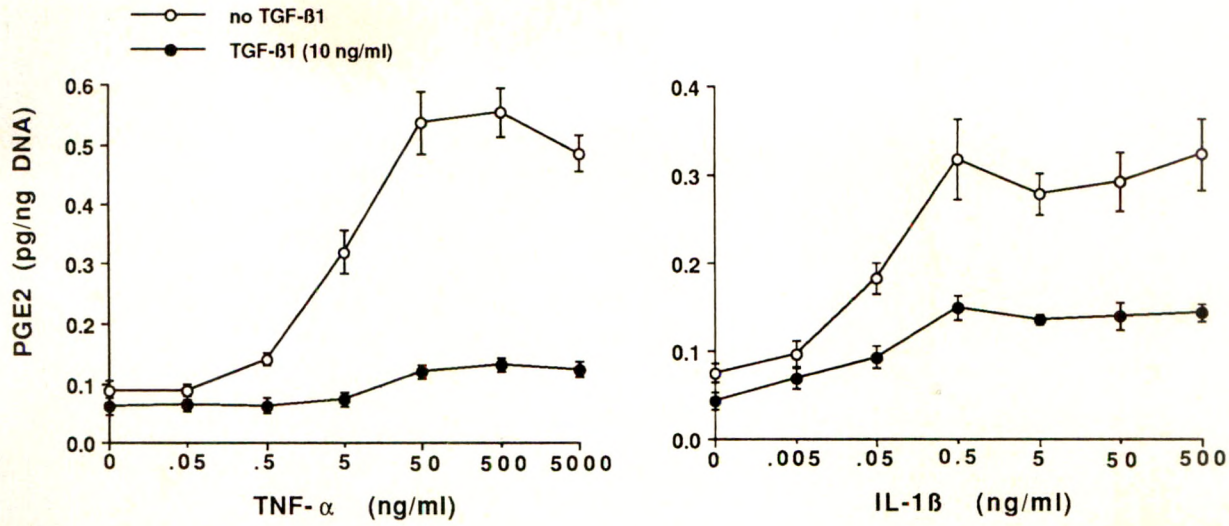


Fig. 1. Effect of TGF-β on amnion cells stimulated with different concentrations of TNF-α or IL-1β. Human amnion cells were treated with indicated concentrations of TNF-α or IL-1β in presence (●) or absence (○) of human TGF-β1 (10 ng/ml). PGE_2 production (picograms per nanogram DNA) was measured after 42-hour incubation. Results are means ± SE (n = 4) of one representative experiment.

their receptors have been identified. TGF-β1 and TGF-β2 are homodimers, and TGF-β1,2 is a heterodimer containing one polypeptide chain each from TGF-β1 and TGF-β2. Most tissues found at the maternal-fetal interface contain TGF-β and express TGF-β messenger ribonucleic acid.[12] In many biologic processes, TGF-β acts as a biologic switch, antagonizing or modifying the action of other growth factors or cytokines.[13] We therefore studied the effect of TGF-β on the cytokine-induced PGE_2 production by human amnion cells in culture. In this report we show that TGF-β suppresses the IL-1– or TNF-stimulated PGE_2 production by amnion cells.

Material and methods

Chemicals. Recombinant human TNF-α (specific activity 4.8×10^7 U/mg, endotoxin content <0.13 endotoxin unit [EU]/mg) and TNF-β (specific activity 2.1×10^8 U/mg, endotoxin 3.1 EU/mg) were generously provided by Genentech, South San Francisco. Recombinant human IL-1β (specific activity 1.8×10^7 U/mg, endotoxin 2.5 EU/mg) was a generous gift from the National Cancer Institute. Recombinant human IL-1α (specific activity 3×10^8 U/mg, endotoxin 0.7 EU/mg) was a kind gift from Hoffmann-La Roche, Inc., Nutley, N.J. The PGE_2 kit was from New England Nuclear, Boston. Human TGF-β1 and porcine TGF-β1, TGF-β2, and TGF-β1,2 were from R&D Systems, Minneapolis.

Preparation and culture of human amnion cells. Human fetal membranes were obtained from normal pregnancies at cesarean sections conducted at term before the onset of labor. Cells from the reflected amnion were isolated as described.[14] The cells were seeded in tissue culture plates at a density of 1.4×10^5 cells/cm² and maintained at 37° C in an atmosphere of air and 5% carbon dioxide in Eagle's minimum essential medium with Earle's salts supplemented with glutamine, 10% fetal calf serum, antibiotics, and antimycotics. After 24 hours the wells were washed with minimum essential medium, and the medium was changed to the culture medium supplemented with cytokines, TGF-β, or the appropriate vehicle. The cells were incubated for 42 hours before the culture media were removed

Table I. Effect of TGF-β on PGE_2 production by amnion cells stimulated with IL-1 or TNF

	PGE_2 production (fractions of control)		*Inhibition (%) in PGE_2 production caused by TGF-β1*
	Without TGF-β1	*With TGF-β1*	
Control	1	0.7 ± 0.1	27 ± 9
IL-1α	25.5 ± 9.6	4.1 ± 1.8	77 ± 4
IL-1β	27.3 ± 10.6	4.4 ± 2.0	75 ± 5
TNF-α	48.4 ± 7.9	9.7 ± 3.1	75 ± 8
TNF-β	9.6 ± 2.6	1.4 ± 0.3	71 ± 9

Amnion cells were treated with IL-1α (6.8 ng/ml), IL-1β (5 ng/ml). TNF-α (50 ng/ml), TNF-β (10 ng/ml), or vehicle (control) in the presence or absence of human TGF-β1 (10 ng/ml). After 42 hours of incubation, the media were removed and analyzed for PGE_2. PGE_2 production is shown as fractions of production in untreated (control) cells; in these cells PGE_2 production was 0.031 ± 0.008 pg/ng DNA. The inhibition in percent caused by TGF-β in each experiment was calculated. The means ± SE of six separate experiments are shown.

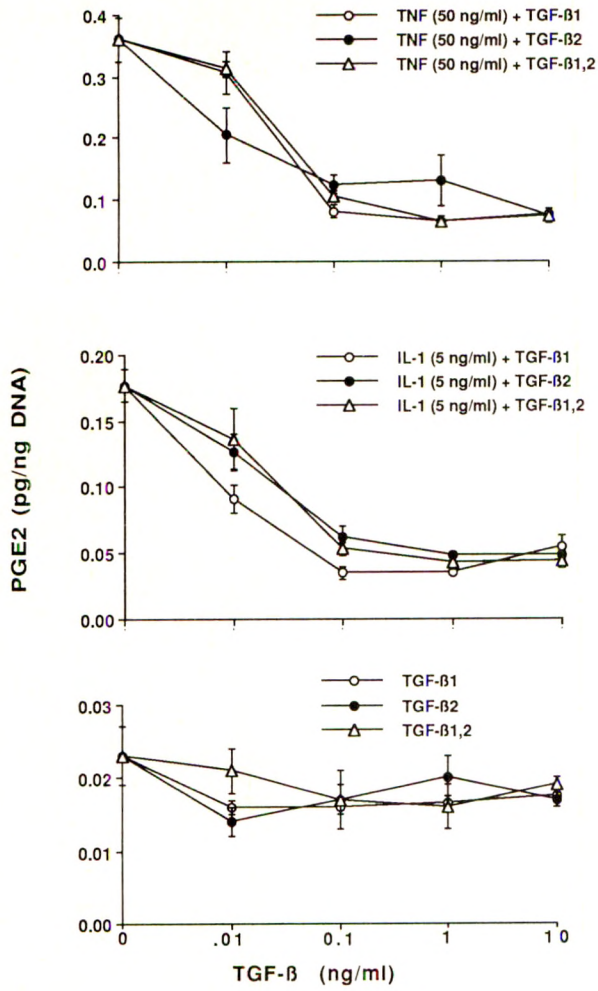


Fig. 2. Comparison of effects of TGF-β1, TGF-β2, and TGF-β1,2 on PGE_2 production by amnion cells. Human amnion cells were treated with TNF-α (50 ng/ml) *(upper panel)*, IL-1β (5 ng/ml) *(middle panel)*, or vehicle *(lower panel)*, in presence of indicated concentrations of porcine TGF-β1 (○), TGF-β2 (●), or TGF-β1,2 (Δ). The PGE_2 production (picograms per nanogram DNA) was measured after 42-hour incubation. Results are means ± SE (n = 4) of one representative experiment.

and then centrifuged at 600 g for 10 minutes to eliminate cell debris. The supernatants were stored at −70° C until assayed for PGE_2. The cells were recovered from the wells with trypsin. More than 90% of the cells excluded trypan blue.

Measurement of PGE_2. PGE_2 was measured by radioimmunoassay directly from the media. The concentrations of PGE_2 in the media incubated in the same conditions but without amnion cells were used as blanks. PGE_2 formation was calculated per nanogram of amnion cell deoxyribonucleic acid (DNA); DNA analysis was done as described.[15] The different treatments with cytokines or TGF-β did not significantly affect the DNA content of the wells.

Statistics. The test used was the Wilcoxon signed-rank test.

Results

Effect of TGF-β on PGE_2 production by amnion cells stimulated with IL-1 or TNF. Human amnion cells were treated with IL-1α (6.8 ng/ml), IL-1β (5 ng/ml), TNF-α (50 ng/ml), TNF-β (10 ng/ml), or vehicle in the presence or absence of human TGF-β1 (10 ng/ml). Because amnion cells do not metabolize PGE_2, the concentration of PGE_2 in the medium at the end of the 42-hour incubation represents the total PGE_2 released during that time.[8] For each cytokine the PGE_2 production was lower in the presence of TGF-β in each of the six separate experiments performed (p = 0.028) (Table I). The decrease caused by TGF-β in the basal PGE_2 production was 27% ± 9%. However, in cells stimulated by IL-1 or TNF, the decrease in total PGE_2 production occurring in the presence of TGF-β1 ranged from 71% ± 9% to 77% + 4% (Table I).

Effect of TGF-β on stimulation of amnion cell PGE_2 production by combination of IL-1 and TNF. Amnion cells were incubated for 42 hours with IL-1β (5 ng/ml), IL-1α (6.8 ng/ml), or TNF-α (5 μg/ml) in the presence or absence of human TGF-β1 (10 ng/ml). IL-1 acts synergistically with TNF to stimulate amnion cell PGE_2 production.[9] Nevertheless, the degree of inhibition due to TGF-β1 was approximately the same as was obtained in cells treated with only one cytokine (Table II). Similar results were obtained with TNF-β (10 ng/ml) instead of TNF-α (results not shown).

Effect of TGF-β as a function of IL-1 and TNF concentration. Amnion cells were incubated with different concentrations of IL-1β (0 to 500 ng/ml) or TNF-α (0 to 5 μg/ml) with or without TGF-β1 (10 ng/ml). The PGE_2 release was assayed after incubation for 42 hours. IL-1β and TNF-α stimulated the PGE_2 production in a concentration-dependent manner; the saturating concentrations for IL-1β and TNF-α were 0.5 and 50 ng/ml, respectively. At each concentration of IL-1β and of TNF-α, TGF-β1 inhibited the PGE_2 production as seen in Fig. 1.

Effect of different concentrations of TGF-β1, TGF-β2, or TGF-β1,2 on PGE_2 production by amnion cells. Cells were treated for 42 hours with IL-1β (5 ng/ml) or TNF (50 ng/ml), together with different concentrations (0 to 10 ng/ml) of porcine TGF-β1, TGF-β2, or TGF-β1,2. The potencies of TGF-β1, TGF-β2, and TGF-β1,2 in inhibiting the PGE_2 production were similar at each concentration tested (Fig. 2). In each case maximal inhibition was obtained with TGF-β concentrations ≥0.1 ng/ml (Fig. 2). The effects of porcine TGF-β1 were indistinguishable from those of human TGF-β1 (data not shown).

In amnion cells not treated with cytokines, TGF-β1,

Table II. Effect of TGF-β1 on amnion cell PGE_2 production stimulated by combination of IL-1 and TNF

	PGE_2 production		*Inhibition caused by TGF-β1 (%)*
	Without TGF-β1	*With TGF-β1*	
Control	0.046 ± 0.002	0.029 ± 0.001	40.0 ± 1.7
IL-1α (6.8 ng/ml)	1.55 ± 0.035	0.237 ± 0.024	84.7 ± 1.6
IL-1β (5 ng/ml)	1.83 ± 0.029	0.195 ± 0.013	89.3 ± 0.8
TNF-α (50 ng/ml)	2.83 ± 0.034	0.312 ± 0.025	89.0 ± 0.8
IL-1α (6.8 ng/ml) + TNF-α (50 ng/ml)	9.006 ± 0.323	1.678 ± 0.051	81.3 ± 0.8
IL-1β (5 ng/ml) + TNF-α (50 ng/ml)	8.671 ± 0.312	1.71 ± 0.077	80.2 ± 1.2

Amnion cells were treated with indicated cytokines or cytokine combinations in presence or absence of human TGF-β1 (10 ng/ml). Total release of PGE_2 (picograms per nanogram DNA) during 42-hour incubation was measured. Data are results (means ± SE, $n = 4$) of one representative experiment.

TGF-β2, and TGF-β1,2 also appeared equipotent, but the degree of inhibition was smaller than that obtained in cells stimulated with cytokines (Fig. 2).

Comment

In this report we show for the first time that TGF-β strikingly opposes the IL-1– or TNF-stimulated PGE_2 production by human amnion cells. It also suppressed the synergistic PGE_2-stimulatory effect of the combination of IL-1 with TNF. The basal PGE_2 production by amnion cells was also inhibited by TGF-β but to a smaller extent.[16] According to the present results, TGF-β may play a role in limiting or terminating the prostaglandin production induced by proinflammatory cytokines in the setting of intrauterine infection.

At all concentrations tested the effects of TGF-β1, TGF-β2, and TGF-β1,2 on amnion cell PGE_2 production were similar. TGF-β1 and TGF-β2 differ from each other by 32-amino-acid substitutions and have 71% sequence identity. In many cell systems TGF-β1 and TGF-β2 are biologically equipotent; however, they show marked differences in certain cells.[10]

In contrast to its effects on amnion cells, TGF-β has a stimulatory effect on the production of prostaglandins in many cells. Thus it stimulates the PGE_2 production in neonatal mouse calvaria[17] and in lung fibroblasts,[18] as well as prostacyclin production in human vascular endothelial cells.[19] In addition, TGF-β potently synergizes with IL-1β and TNF-α in stimulating the PGE_2 production in rat renal mesangial cells.[20]

TGF-β–like activity is present in many cells at the fetomaternal interface.[12] TGF-β1 antibody reacts with cells in the chorion and with extracellular matrix components in the placenta and uterus.[21] TGF-β1 messenger ribonucleic acid is present in the placenta, decidua, and placental membranes.[12] A TGF-β2–like factor has been described in murine amniotic fluid.[22] High levels of TGF-β2 messenger ribonucleic acid are present in the uterus of the pregnant mouse; they fall rapidly as birth approaches.[22]

Besides the observed effect of TGF-β on PGE_2 synthesis, there is additional evidence suggesting the importance of TGF-β to the maintenance of pregnancy. TGF-β is a potent immunosuppressant both in vivo and in vitro.[23] Murine decidual cells secrete an immunosuppressive TGF-β2–like molecule.[24] TGF-β may be essential in the maintenance of pregnancy by preventing rejection of the fetus.[24]

TGF-β induces the accumulation of extracellular matrix by several mechanisms. It stimulates the synthesis of many extracellular matrix components.[25] It also enhances the adhesion of extracellular matrix on cells.[26] In addition, TGF-β up-regulates inhibitors of extracellular matrix–degrading enzymes[27,28] and inhibits enzymes (such as collagenase) that degrade extracellular matrix components.[28] The effect of TGF-β on cell adhesion receptors may be of importance in the attachment of the fetoplacental unit to the uterine wall. Because of its effects on extracellular matrix, TGF-β has the potential of inhibiting processes required for both term and preterm labor. These include softening and dilatation of the cervix, which is associated with a decrease in collagen content and an increase in collagenolytic activity.[29] The biologic activity of TGF-β and its regulation during pregnancy remain to be clarified.

From the current results we propose that TGF-β plays an important role in the modulation of processes that regulate the initiation of preterm labor. In cases of intrauterine inflammation, increased production of cytokines threaten pregnancy. The continuation of pregnancy may depend on the balance of the activities of labor-promoting agents, such as IL-1 and TNF, and of agents, such as TGF-β, that favor the continuation of pregnancy. It remains to be seen whether TGF-β has therapeutic potential in the prevention of preterm labor.

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Mutations of the Ki-*ras* oncogene in endometrial carcinoma

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OBJECTIVE: The purpose of this study was to assess the extent of involvement of the *ras* oncogene in endometrial carcinoma.

STUDY DESIGN: Genomic deoxyribonucleic acid from 30 samples of endometrial carcinoma was examined for point mutations in codons 12, 13, and 61 from the Ha-*ras*, Ki-*ras*, and N-*ras* genes by means of the polymerase chain reaction, slot-blotting, and deoxyribonucleic acid sequencing procedures.

RESULTS: An apparent somatic mutation of Ki-*ras* codon 12 in one of 10 paraffin-embedded tumors was confirmed by deoxyribonucleic acid sequence analysis. Two of 20 frozen endometrial carcinoma specimens were also shown to contain a point mutation in Ki-*ras* codon 12. No correlation between *ras* mutation and a number of histologic or clinical parameters was observed.

CONCLUSIONS: These data suggest a potential role for Ki-*ras* codon 12 mutations in the development of some (10%) endometrial cancers. (Am J Obstet Gynecol 1992;167:227-32.)

Key words: Endometrial carcinoma, oncogene, *ras*, polymerase chain reaction

Carcinoma of the uterine endometrium is the most frequently diagnosed gynecologic malignancy in the United States, but few data on the molecular genetic features of this tumor exist. We believe that a series of genetic alterations involving both protooncogenes and tumor suppressor genes plays a role in the development of endometrial carcinomas as it does in other human cancers.[1] The variable nature of these genetic events can be noted in the clinical observation of two distinct forms of endometrial carcinoma.[2, 3] Type I tumors are frequently associated with a history of unopposed estrogen exposure or other hyperestrogenic condition such as obesity. They occur in perimenopausal women, are typically of low grade and good prognosis, and are often associated with or preceded by endometrial hyperplasia. In contrast, type II tumors, which have a poorer prognosis, appear unrelated to estrogenic stimulation, manifest at a later age (>60), and are generally of higher grade and/or uncommon histologic subtype. The clustering of endometrial carcinoma in certain families[4] and the occurrence in patients with multiple primary tumors (especially those of the colon)[4-7] suggest genetic transmission of endometrial cancer risk.

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The potential role of protooncogenes in the cause of endometrial carcinoma has only recently been addressed, unlike the etiologic factors of the more well-characterized female cancers of the breast, ovary, and uterine cervix. Amplification and/or overexpression of the c-*erb*B2 protooncogene may occur in a subset of tumors, although the extent of this phenomenon is still unclear.[8-10] In addition, the inappropriate expression of colony stimulating factor-1 and its receptor, the c-*fms* protooncogene, has been implicated as a potential autocrine growth stimulatory pathway in endometrial carcinoma.[11, 12]

Activating point mutations in members of the *ras* gene family represent the most frequent oncogene abnormality in human tumors.[13] Several recent reports suggest that *ras* mutations may play a role in some endometrial carcinomas; specifically, a total of 6 of 12 human endometrial carcinoma cell lines in two studies[10, 14] and 13 of 41 primary tumors in three studies[14-16] exhibit point mutations in Ki-*ras* codon 12. In the three previous studies of primary tumor specimens, however, a relatively small number of cases were examined in each study (10, 12, and 19, respectively), and a majority of the mutations (11 of 13) were found in endometrial carcinomas from Japan[14, 16]; the remaining two were from Australia.[15] Additional studies are needed to define the extent of involvement of the *ras* gene family in endometrial carcinomas from the United States, where the incidence of endometrial carcinoma is the highest in the world and where a majority of tumors are of the type I variety, in contrast to Japan, where the incidence of endometrial carcinoma is the lowest in the world and where a significantly higher fraction of tumors are of the type II classification.[17]

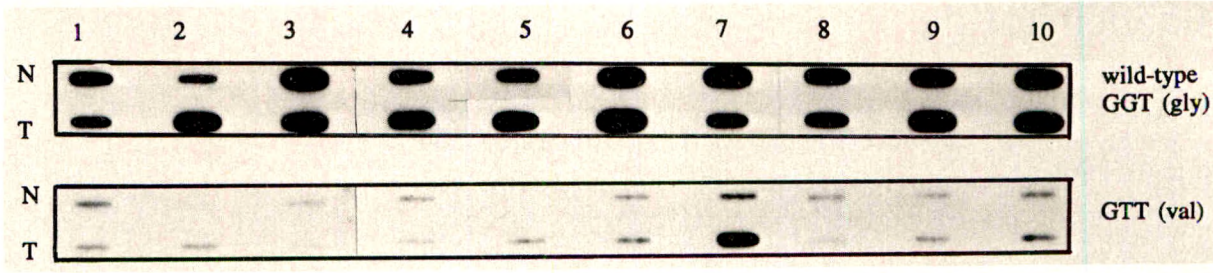


Fig. 1. Somatic mutation of Ki-*ras* codon 12 as detected by oligonucleotide hybridization with polymerase chain reaction–amplified DNA. DNA samples from normal *(N)* and tumor *(T)* tissue from 10 cases of formalin-fixed, paraffin-embedded endometrial carcinoma were analyzed for all possible activating point mutations in codons 12, 13, and 61 of Ha-*ras*, Ki-*ras*, and N-*ras* genes. Tumor but not normal tissue DNA from case 7 exhibited positive hybridization with mutant probe GTT for Ki-*ras* codon 12.

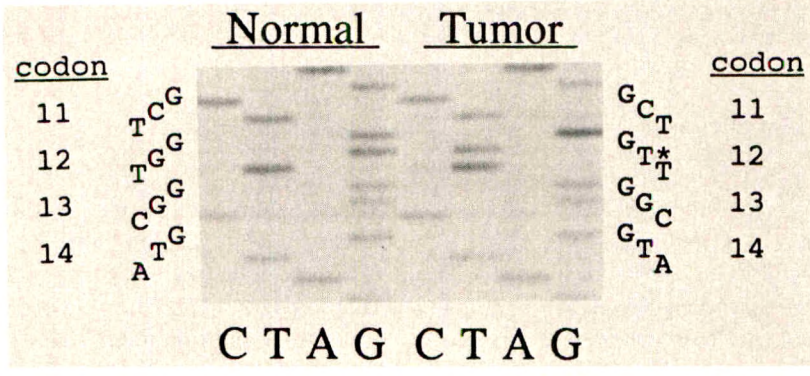


Fig. 2. Autoradiogram of sequencing gel for endometrial carcinoma case 7 in Fig. 1. The slot-blotting data indicating a point mutation of Ki-*ras* codon 12 was confirmed by DNA sequence analysis; clone of polymerase chain reaction–amplified DNA from normal tissue contained wild-type sequence GGT *(gly)*, and clone of polymerase chain reaction–amplified DNA from tumor tissue contained mutant sequence GTT *(val)*. *Asterisk*, Mutant codon.

The primary goal of this study was to examine a sufficiently large sample of endometrial carcinomas from the United States so that the extent of involvement of the *ras* oncogene in tumors from this population could be assessed. We then wished to determine if the presence of *ras* mutations correlated with parameters such as histologic factors, grade, stage, and receptor status.

Material and methods

Tumor samples and DNA isolation. Ten formalin-fixed, paraffin-embedded endometrial carcinoma specimens from the year 1990 were retrieved at random from the surgical pathology archives at the North Carolina Memorial Hospital. Genomic deoxyribonucleic acid (DNA) was extracted and was purified with a modification of a procedure described by Goelz et al.[18] Histopathologic sections 10 μm thick were cut and placed on untreated glass slides; 10 to 30 sections were cut for each tumor. One slide stained with hematoxylin and eosin stain was used to discriminate areas of tumor from normal tissue, and the bottom of each unstained slide was marked accordingly. Normal and tumor tissue were scraped separately from the slides with a straight-edged scalpel blade, were minced into small pieces, and were collected in separate 15 ml polypropylene conical tubes. DNA was then extracted and purified as described,[18] with the exception that samples were not sheared through a syringe needle (this step was found to decrease the size of genomic DNA without increasing the yield appreciably).

Twenty fresh-frozen endometrial carcinoma samples, obtained between 1989 and 1991 at the Duke University Medical Center, were processed for genomic DNA by means of standard procedures.[19]

Polymerase chain reaction and slot-blotting procedures. DNA was screened for *ras* mutations by means of polymerase chain reaction amplification, slot-blotting, and hybridization with oligodeoxynucleotides, as described previously.[10, 20] A panel of 66 oligonucleotide probes that were specific for wild-type or mutant sequences at codons 12, 13, and 61 of the Ha-*ras*,

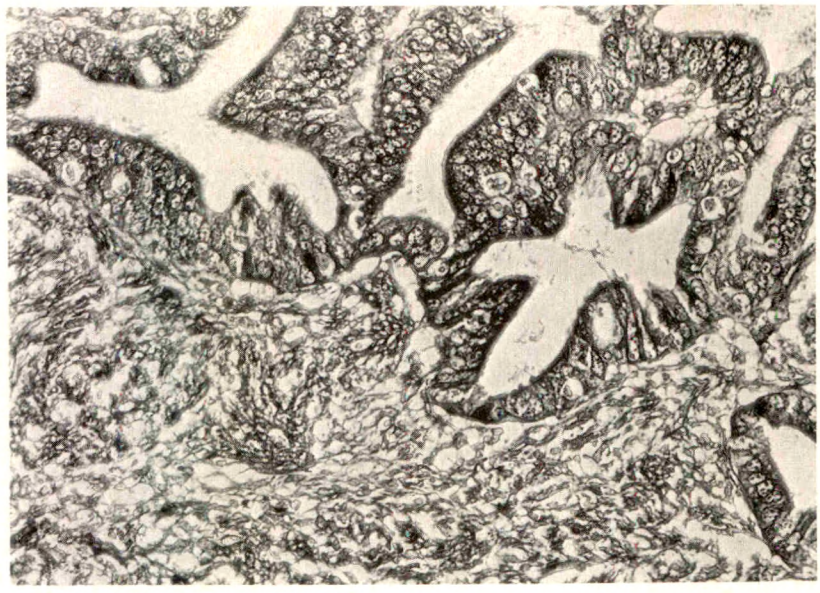


Fig. 3. Histologic photomicrograph of stained section from endometrial carcinoma case 7. The junction of carcinoma with normal myometrium illustrates the distinction of "tumor" versus "normal" in separating paraffinized tissue for DNA extraction; glandular organization indicates moderately differentiated endometrioid adenocarcinoma. (Hematoxylin and eosin. Original magnification ×200.)

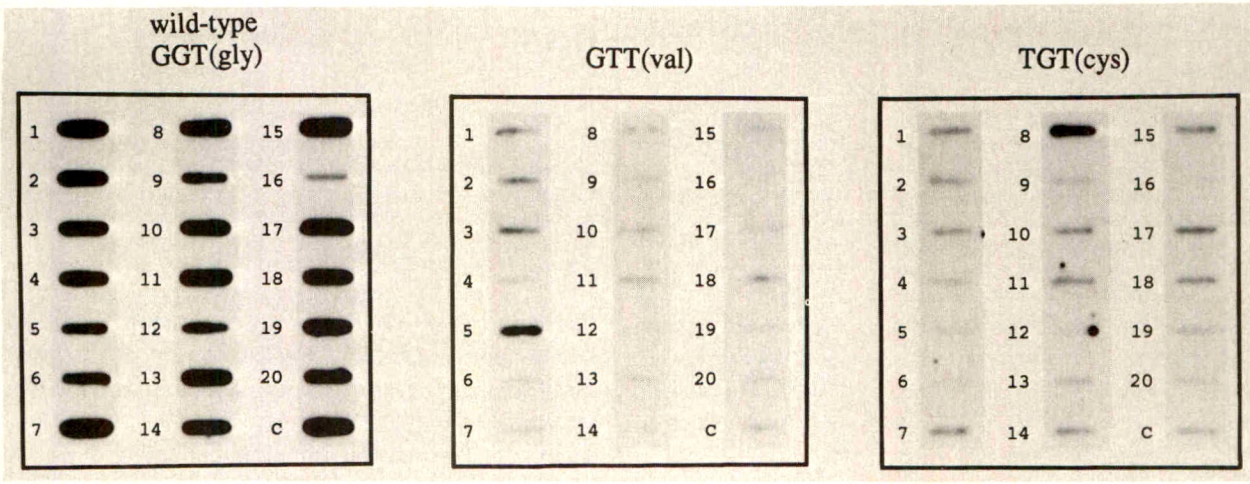


Fig. 4. Mutation of Ki-*ras* codon 12 in fresh-frozen endometrial carcinoma samples as detected by oligonucleotide hybridization with polymerase chain reaction–amplified DNA. Cases 5 and 8 exhibit mutations of wild-type GGT *(gly)* to GTT *(val)* and TGT *(cys)*, respectively.

Ki-*ras*, and N-*ras* genes was used to analyze DNA from normal and tumor tissue from each of the 10 archival endometrial carcinoma cases. The 20 fresh-frozen cases were analyzed only for mutations at Ki-*ras* codon 12. Before slot-blotting was performed, all samples were analyzed by agarose gel electrophoresis to ensure success of the polymerase chain reaction; in addition, all experiments were carried out with positive controls (DNA from previously characterized endometrial carcinoma cell lines[10]) and negative controls (plasmid DNA).

Sequence analysis. For cases determined to be harboring probable *ras* mutations, polymerase chain reaction amplified DNA fragments were subcloned and sequenced with the dideoxy chain termination method. After amplification, polymerase chain reaction products were purified by centrifugation in a Centricon-30 microconcentrator (Amicon, Danvers, Mass.) and were subcloned into the *Sma*I site of pBluescript SKII$^+$ (Stratagene, La Jolla, Calif.). Clones were sequenced with polymerase chain reaction primers according to the manufacturer's protocol for Sequenase version 2.0. Se-

Table I. Clinicopathologic parameters and *ras* mutations

Case No.	Histologic factors	FIGO grade	Surgical stage	Uterine invasion	Steroid receptors		Ki-ras/12
					Estrogen	Progesterone	
Archival							
1	EA	2	NA	2		NA	wt
2	MA	1	IA	0	+	+	wt
3	EA	1	IC	2	+	+	wt
4	EA	2	II	2	+	+	wt
5	AA	2	IA	1	+	+	wt
6	EA	1	IC	2	+	+	wt
7	EA	2	IB	1	+	+	mut
8	EA	2	IB	1	+	+	wt
9	AS	2	IC	2	+	+	wt
10	EA	2	IA	1	+	+	wt
Frozen							
1	EA	1	IIIA	3	+	+	wt
2	EA	3	IVB	3		NA	wt
3	PC	2	IIIA	2	+	−	wt
4	EA	2	IIIA	1	−	−	wt
5	EA	3	IIIC	2	−	−	mut
6	EA	1	IIIA	1	+	+	wt
7	EA	2	IC	3	+	+	wt
8	EA	2	IB	1	−	+	mut
9	AS	2	IIIC	3	−	−	wt
10	EA	2	IC	2	−	+	wt
11	EA	2	IVB	3	+	−	wt
12	EA	2	IC	3	+	+	wt
13	EA	1	IA	0	−	+	wt
14	AS	3	IC	3	+	−	wt
15	CC	3	IVB	1	−	−	wt
16	EA	2	IC	3	+	+	wt
17	EA	2	IB	1	−	+	wt
18	EA	2	IC	3		NA	wt
19	EA	2	IIIA	1	−	+	wt
20	EA	2	IB	1	+	+	wt

FIGO, International Federation of Gynecology and Obstetrics; *EA*, endometrioid adenocarcinoma; *MA*, mucinous adenocarcinoma; *AA*, adenoacanthoma; *AS*, adenosquamous carcinoma; *PC*, papillary carcinoma; *CC*, clear cell carcinoma. For uterine invasion: *0*, none; *1*, inner third; *2*, middle third; *3*, outer third. For steroid receptors: Estrogen/progesterone; +, >10 fmol/mg. For Ki-*ras*: *wt*, wild-type; *mut*, mutant. *NA*, not available.

quencing reactions were subjected to electrophoresis in 7 mol/L urea–6% polyacrylamide gels at 65 W constant power, were fixed in 10% methanol–10% acetic acid, were dried, and were exposed to Kodak X-Omat AR x-ray film for 1 to 3 days.

Results

All 10 of the formalin-fixed, paraffin-embedded tissue specimens analyzed yielded both normal and tumor DNA of sufficient quality and quantity for subsequent genetic analysis. The paraffin blocks invariably contained enough tumor and normal tissue so that from 30 to several hundred micrograms of genomic DNA was isolated from all cases. For each sample, the DNA migrated on ethidium bromide–stained agarose gels as a single high-molecular-weight band (>23 kb) with a smear of degraded, lower-molecular-weight DNA. Although the tumors described in this paper were all <2 years old, we have obtained DNA of sufficient quality for polymerase chain reaction procedures from fixed and embedded human tumors as old as 20 years.

DNA from these 10 cases was examined by means of polymerase chain reaction and slot-blotting procedures for all possible activating point mutations of codons 12, 13, and 61 of the Ha-*ras*, Ki-*ras*, and N-*ras* genes. One tumor contained an apparent mutation in one allele of Ki-*ras* codon 12 that was not evident in the surrounding normal tissue from this case, which indicated the somatic nature of this event (Fig. 1). A GGT→GTT (Gly→Val) transversion in codon 12 of Ki-*ras* was confirmed by sequencing of the polymerase chain reaction product; only the wild-type sequence was evident in DNA from the normal tissue of this case (Fig. 2). This tumor was classified with respect to histologic factors as a moderately differentiated endometrioid adenocarcinoma; a hematoxylin and eosin stained photomicrograph of the tumor and myometrial border is shown in Fig. 3.

Twenty additional specimens of fresh-frozen endometrial carcinoma were examined for mutations in Ki-*ras* codon 12. Two of these tumors, a moderately differentiated endometrioid adenocarcinoma and a

poorly differentiated endometrioid adenocarcinoma, exhibited activating point mutations: One contained the same GGT→GTT transversion described above, and the other contained a GGT→TGT (Gly→Cys) transversion in the first nucleotide of this codon (Fig. 4). These mutations were also heterozygous in nature; the tumors contained both a mutant and wild-type allele.

The mutations and histopathologic data for the 30 tumors described in this report are summarized in Table I in which no correlation between Ki-*ras* mutation and any of the clinical, pathologic, or histologic criteria was reported.

Comment

These data indicate that somatic point mutation of a *ras* oncogene occurs at a significant but relatively low (10%) frequency in endometrial carcinomas in the United States. Two previous studies[14, 16] showed that *ras* mutation occurs in endometrial carcinomas from Japan at about a threefold higher frequency than that observed here. These data suggest that *ras* mutation may be a more frequent occurrence in the type II tumors more prevalent in Japan than in the type I estrogen-related tumors that are more prevalent in the United States. The higher incidence of *ras* mutation that we had observed in endometrial carcinoma cell lines[10] may reflect spontaneous mutation and/or clonal selection of mutant *ras* genes during establishment and culture of these cells. All the existing data, however, implicate Ki-*ras* codon 12 as the most frequently altered *ras* site in endometrial carcinoma.

The *ras* family of protooncogenes encodes a unique form of GTPase molecule that appears to play a central role in cell proliferation as a result of its regulation of signal transduction. Point mutations in codons 12, 13, or 61 appear to inactivate this GTPase activity, which leaves the mutant protein in a constitutively activated state.[21] A large number and variety of human tumors contain point-mutated *ras* oncogenes,[13] which suggest a generalized role for *ras* in the proliferation of diverse tissues. Other solid tumors that contain mutations of Ki-*ras* at significant frequencies include carcinomas of the lung (20% to 30%), pancreas (70% to 90%), thyroid (20% to 60%), and colon (40% to 50%).[13] Endometrial carcinomas, like those of the lung, pancreas, and colon, exhibit a predominance of Ki-*ras* codon 12 mutations; thus a similar role for Ki-*ras* in the regulation of cell proliferation in these tissues or perhaps similar molecular pathways of neoplastic development or progression in these tissues.

Mutated *ras* genes have been strongly implicated in experimental chemical carcinogenesis[22]; this suggests that *ras* mutations in endometrial tumors may arise from chemical (including hormonal) mutagenesis. Alternatively, because cyclical cell proliferation is a prominent and somewhat unique feature of the mammalian endometrium, spontaneous DNA aberrations would be subject to a greater probability of conversion to permanent mutations in this tissue. Estrogen may thus act indirectly to stimulate tumorigenesis, by increasing the proliferative index of endometrial tissue.[23] The Ki-*ras* mutations reported in this paper whether they were spontaneous or chemically induced, did not appear to be associated with any histopathologic or clinical criteria. This suggests a sporadic induction and random distribution in endometrial carcinoma.

To extend these findings it will be necessary to examine a larger number of tumors, especially those of the more uncommon histologic (type II) variants. In particular, it would be instructive to compare a larger number of tumor samples from the United States with those from Japan, where estrogen-related risk factors and the incidence of endometrial carcinoma are much lower than those in the United States.[3, 17] That comparison might indicate a dramatically different ratio of type II to type I tumors. In addition, it would be of interest to analyze premalignant hyperplastic lesions of the endometrium for *ras* mutations to determine if that mutation is an early event in the development of some endometrial cancers as it is in colon carcinoma.

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Effect of maternal oxygen administration on fetal oxygenation during graded reduction of umbilical or uterine blood flow in fetal sheep

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OBJECTIVE: Effects of maternal oxygen administration on fetal blood gases and on oxygen delivery and consumption during reduced uterine and reduced umbilical blood flows were examined.
STUDY DESIGN: In eight pregnant sheep (gestational age 133 ± 4 days) flow transducers were applied to a uterine and the common umbilical artery. Graded reductions in uterine and umbilical blood flows were achieved by a hypogastric artery snare and a balloon cuff encircling the umbilical cord. Fetal femoral arterial and umbilical venous oxygen contents and flows were measured at varying flow reductions with the ewe breathing air or oxygen.
RESULTS: During 75% reduction in umbilical blood flow maternal oxygen administration significantly increased fetal oxygen delivery (6.4 ± 2.5 to 7.7 ± 2.3 ml/min/kg) and oxygen consumption (4.3 ± 1.2 to 5.0 ± 0.8 ml/min/kg). With similar reduction of uterine flow oxygen administration increased oxygen delivery from 8.3 ± 2.4 to 12.3 ± 3.6 and oxygen consumption from 3.3 ± 0.8 to 4.7 ± 1.6 ml/min/kg.
CONCLUSION: Maternal oxygen inhalation improves fetal oxygenation during umbilical but especially during uterine blood flow reduction. (Am J Obstet Gynecol 1992;167:233-9.)

Key words: Umbilical blood flow, fetal oxygen delivery, fetal oxygen consumption

The effect of maternal oxygen administration during labor on fetal oxygenation is controversial. In one clinical study maternal oxygen administration increased fetal arterial P_{O_2} from 15 to 21 mm Hg.[1] In long-term, growth-retarded human fetuses maternal oxygen administration increased umbilical venous P_{O_2}.[2] On the other hand, it has been argued that maternal oxygen administration will have no major effect on fetal oxygenation because maternal arterial blood is almost completely saturated with oxygen; therefore, increasing the maternal arterial P_{O_2} above 100 mm Hg will increase only the amount of oxygen physically dissolved, which is relatively small compared with that attached to hemoglobin.[3]

During labor fetal oxygenation may need to be improved for reduction of uterine or umbilical blood flow. In fetal sheep maternal oxygen administration during reduction of umbilical blood flow improved fetal oxygenation by a modest degree.[4] Uterine blood flow may be markedly reduced in long-term, growth-retarded sheep fetuses, and maternal oxygen administration resulted in a significant improvement of fetal oxygenation.[5] However, the effect of maternal oxygen administration on fetal oxygenation during reductions of uterine blood flow has not been investigated systematically.

The aim of the present study was to assess the effect of maternal oxygen administration on fetal blood gases, oxygen delivery, and oxygen consumption during graded reductions of uterine blood flow. Furthermore, the effect of maternal oxygen administration during reduction of uterine blood flow was compared with that during reduction of umbilical blood flow of similar degree.

Material and methods

Animal preparation. Animal care and experimental procedures were approved by the Committee on Animal Research at the University of California, San Francisco.

Eight mixed-breed Western ewes with time-dated singleton gestations of 133 ± 4 days at the time of study (term 145 days) were fasted 24 hours, given spinal anesthesia with 4 ml 1% tetracaine hydrochloride (Pontocaine, Winthrop Laboratories, Murray Hill Station, N.Y.), and placed supine on the operating table. Polyvinyl catheters (inner diameter 0.05 inches) were inserted into pedal branches of a maternal femoral artery and vein and advanced to the descending aorta and

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Table I. Effect of maternal oxygen administration on fetal blood gases during graded reductions in umbilical blood flow

Variable	*Condition*	*Baseline*	*Umbilical blood flow reduction*		
			30%	*50%*	*75%*
Fetal descending aorta					
Hemoglobin (gm/dl)	Room air	12.0 ± 1.5	12.4 ± 1.9	13.0 ± 1.8	13.7 ± 2.0
	Oxygen	11.6 ± 1.5	12.1 ± 1.7	12.6 ± 1.6	13.7 ± 1.9*
Oxygen saturation (%)	Room air	54.7 ± 5.6	45.9 ± 9.0	33.7 ± 9.9	20.7 ± 9.3
	Oxygen	72.5 ± 3.5	61.9 ± 12.5	44.4 ± 14.8	28.2 ± 13.0*†‡
Umbilical vein					
Hemoglobin (gm/dl)	Room air	11.7 ± 1.5	12.1 ± 1.8	12.6 ± 1.7	13.4 ± 2.0
	Oxygen	11.3 ± 1.4	11.9 ± 1.6	12.3 ± 1.6	13.4 ± 1.8*
Oxygen saturation (%)	Room air	79.1 ± 6.8	79.0 ± 8.0	74.9 ± 4.6	70.9 ± 7.0
	Oxygen	98.2 ± 3.9	96.6 ± 4.5	91.9 ± 2.7	88.4 ± 2.2†
pH	Room air	7.41 ± 0.06	7.39 ± 0.08	7.38 ± 0.06	7.34 ± 0.07
	Oxygen	7.37 ± 0.06	7.36 ± 0.07	7.34 ± 0.07	7.27 ± 0.09*†
P_{CO_2} (mm Hg)	Room air	45.0 ± 5.0	47.4 ± 5.7	48.8 ± 3.4	51.4 ± 4.5
	Oxygen	51.2 ± 8.8	51.3 ± 5.0	53.0 ± 6.7	54.4 ± 7.3*†
P_{O_2} (mm Hg)	Room air	31.3 ± 4.1	32.0 ± 2.0	29.8 ± 1.0	32.1 ± 9.5
	Oxygen	57.3 ± 9.7	53.9 ± 8.2	45.2 ± 6.9	45.8 ± 10.7†‡

Data are mean ± SD from five experiments.
*Significant effect of blood flow reduction.
†Significant effect of oxygen.
‡Significant interaction between blood flow reduction and oxygen.

inferior vena cava, respectively. A continuous intravenous infusion of 0.9% saline solution (500 to 1000 ml) was administered to the ewe throughout the surgical procedure. Ketamine hydrochloride, 50 to 100 mg, was injected intravenously as necessary to maintain sedation. The ewe's abdomen was opened in the midline, and a snare was placed around the common segment of the uterine artery so that uterine blood flow could be controlled. In addition, a precalibrated electromagnetic flow transducer of appropriate size was placed around the main uterine artery supplying the pregnant horn.

The fetal hindlimbs were exposed through a small uterine incision. Anesthesia for the placement of all fetal catheters was provided locally with 0.5% lidocaine hydrochloride and systemically with 15 to 25 mg intramuscular ketamine hydrochloride given to the fetus. Polyvinyl catheters (inner diameter 0.03 inches) were inserted through pedal branches into the descending aorta and the inferior vena cava. A 3.5 Fr multiple sidehole catheter was inserted through a cotyledonary tributary into one of the two main umbilical veins. After placing an inflatable cuff occluder around the umbilical cord, an amniotic catheter was inserted to lie adjacent to the fetal trunk. Through a separate uterine incision, the common umbilical artery was isolated by a retroperitoneal approach as described previously.[6] The diameter of the common umbilical artery was measured, ranging from 4.5 to 5.5 mm, and a precalibrated electromagnetic flow transducer of appropriate size was placed around it.

The fetal and uterine incisions were closed, and amniotic fluid lost during surgery was replaced with warm saline solution. All catheters were filled with heparin (1000 IU/ml) and plugged. Together with the transducer cables of the flow probes and the snare, they were then directed subcutaneously to the ewe's flank, exteriorized, and stored in a vinyl pouch. On the day of surgery and each day afterward the ewe received 2 million IU penicillin G and 100 mg gentamicin, half intravenously and half into the amniotic cavity. Catheters were flushed daily and refilled with heparin.

Experimental protocol and measurements. Experiments were performed 1 to 3 days after surgery while the ewe stood quietly in a study cage. Pressures in the fetal descending aorta, umbilical vein, inferior vena cava, and amniotic cavity were measured with carefully calibrated Statham P23Db pressure transducers (Gould Inc., Oxnard, Calif.). Amniotic pressure was used as the zero pressure reference. Fetal heart rate was measured with a cardiotrachometer triggered by the arterial pulse pressure. Readings of uterine and umbilical blood flow were made on Statham SP 2202 electromagnetic blood flow meters. Mean blood flows, fetal heart rate, and pressures were recorded continuously on an Astro-Med MT-8800 direct writing recorder (West Warwick, R.I.).

Baseline blood samples were withdrawn simultaneously from the fetal descending aorta and the umbilical vein while the ewe was breathing room air or 100% oxygen for at least 15 minutes. The oxygen was administered to the ewe through a loosely fitting plastic bag at a rate of 15 L/min. Blood gases and pH were

Table II. The effect of maternal oxygen administration on fetal oxygenation during graded reductions in umbilical blood flow

Variable	*Condition*	*Baseline*	*Umbilical blood flow reduction*		
			30%	*50%*	*75%*
Umbilical blood flow (% of control)	Room air	100	71 ± 6	48 ± 5	25 ± 6
	Oxygen	100	71 ± 7	50 ± 8	25 ± 5*
Umbilical blood flow (ml/min/kg)	Room air	194 ± 19	137 ± 10	94 ± 15	49 ± 14
	Oxygen	190 ± 19	135 ± 14	95 ± 18	48 ± 10*
Umbilical vein oxygen (ml oxygen/dl)	Room air	12.4 ± 1.5	12.8 ± 2.0	12.7 ± 1.6	12.7 ± 1.4
	Oxygen	15.0 ± 1.3	15.5 ± 2.3	15.3 ± 2.2	15.9 ± 1.9†
Descending aorta oxygen (ml oxygen/dl)	Room air	8.8 ± 1.2	7.7 ± 1.7	6.0 ± 2.3	4.0 ± 2.2
	Oxygen	11.3 ± 1.4	10.2 ± 2.7	7.8 ± 3.3	5.5 ± 3.0*†‡
Oxygen delivery (ml oxygen/min/kg)	Room air	24.2 ± 4.6	17.7 ± 4.0	12.0 ± 3.2	6.4 ± 2.5
	Oxygen	28.6 ± 5.0	21.1 ± 5.1	14.8 ± 4.9	7.7 ± 2.3*†‡
Increase in oxygen delivery (ml oxygen/min/kg)		4.4 ± 1.1	3.4 ± 1.4	2.7 ± 1.7	1.3 ± 0.3§
Oxygen consumption (ml oxygen/min/kg)	Room air	7.0 ± 1.6	7.1 ± 1.8	6.2 ± 1.1	4.3 ± 1.2
	Oxygen	6.9 ± 0.8	7.1 ± 1.4	7.0 ± 1.2	5.0 ± 0.8*
Increase in oxygen consumption (ml oxygen/min/kg)		−0.1 ± 0.9	0.0 ± 0.8	0.8 ± 0.6	0.7 ± 0.6

Data are mean ± SD from five experiments.
*Significant effect of blood flow reduction.
†Significant effect of oxygen.
‡Significant interaction between blood flow reduction and oxygen.
§$p < 0.05$ compared with baseline.

measured by a Corning 175 Blood Gas Analyzer (Medfield, Mass.). Hemoglobin concentration and oxygen saturation were measured photometrically in duplicate (Hemoximeter OSM 2, Radiometer, Copenhagen).

After baseline blood samples were obtained, either umbilical or uterine blood flow was reduced in a stepwise fashion. We aimed for 30%, 50%, and 75% reductions of blood flow. At least 10 minutes after the flow remained stable at each grade of reduction, a 0.6 ml blood sample was obtained from the descending aorta and umbilical vein, and fetal heart rate, blood pressures, and blood flows were measured. Measurements were then repeated while the ewe breathed 100% oxygen for 15 minutes. Between each grade of reduction we waited at least 20 to 30 minutes or until pressures and blood flows had returned to baseline values. On each day only one type of blood flow reduction was performed; each type of blood flow reduction was performed only once per animal.

At the end of the studies the ewe was killed with an overdose of pentobarbital sodium (Euthanasia-6, Veterinary Laboratories, Lexena, Kan.) followed by an intravenous injection of 10 ml saturated potassium chloride solution. The fetus was weighed and the correct positions of the catheters, flow probes, and snare were confirmed.

Calculations. The oxygen content in the umbilical vein and the descending aorta, fetal oxygen delivery, and fetal oxygen consumption were calculated as follows:

$$\text{Oxygen content (ml/dl)} = 0.0134 \times \text{Hemoglobin (gm/dl)} \times \text{Oxygen saturation (\%)}$$

$$\text{Oxygen delivery (ml/min)} = \text{Oxygen content}_{UV} \text{ (ml/dl)} \times \text{Placental blood flow (ml/min)}/100$$

$$\text{Oxygen consumption (ml/min)} = \text{Oxygen content}_{UV-DA} \text{ (ml/dl)} \times \text{Placental blood flow (ml/min)}/100$$

where UV is umbilical vein and DA is descending aorta.

Statistical analysis. Data analysis was performed with a computerized statistical program (SuperANOVA, Abacus Concepts, Berkeley, Calif.). Data are expressed as mean ± SD. The effect of blood flow reduction and maternal oxygen administration was analyzed by an analysis of variance for repeated measurements with two within factors. Furthermore, the program tested whether a significant interaction between the two within factors (blood flow reduction and maternal oxygen administration) was present. No post hoc multiple comparison testing was performed because only within-factors and no between-factors variations were present. For all tests, after degree of freedom adjustment, $p < 0.05$ was considered statistically significant.

Results

Fetal weight averaged 3.6 kg (range 3.0 to 4.5 kg). At baseline, while the ewe was breathing room air, fetal blood gases, umbilical blood flow, oxygen delivery, and oxygen consumption were within the normal range of values measured in our laboratory (Tables I to IV).

At baseline maternal oxygen administration increased umbilical venous oxygen saturation to nearly 100%. Because umbilical blood flow did not change, maternal oxygen administration increased fetal oxygen delivery by about 4.5 ml/min/kg, representing a 20%

Table III. The effect of maternal oxygen administration on fetal blood gases during graded reductions in uterine blood flow

Variable	*Condition*	*Baseline*	*Uterine blood flow reduction*		
			30%	*50%*	*70%*
Fetal descending aorta					
Hemoglobin (gm/dl)	Room air	11.3 ± 2.2	11.1 ± 2.3	11.5 ± 2.5	11.9 ± 2.3
	Oxygen	11.0 ± 2.1	11.0 ± 2.2	11.1 ± 2.2	12.0 ± 2.5*
Oxygen saturation (%)	Room air	54.7 ± 12.9	46.8 ± 15.1	31.0 ± 13.1	14.8 ± 6.4
	Oxygen	69.7 ± 5.1	59.7 ± 13.5	49.5 ± 15.3	21.4 ± 9.1*, †
Umbilical vein					
Hemoglobin (gm/dl)	Room air	10.9 ± 2.3	10.9 ± 2.2	11.2 ± 2.5	11.7 ± 2.5
	Oxygen	10.6 ± 2.0	10.8 ± 2.1	10.8 ± 2.2	11.7 ± 2.5*
Oxygen saturation (%)	Room air	79.9 ± 12.9	71.0 ± 15.5	50.9 ± 16.3	25.0 ± 9.3
	Oxygen	95.7 ± 3.3	85.0 ± 12.6	72.0 ± 14.4	35.1 ± 12.8*, †
pH	Room air	7.41 ± 0.02	7.39 ± 0.02	7.36 ± 0.02	7.29 ± 0.05
	Oxygen	7.36 ± 0.02	7.33 ± 0.03	7.31 ± 0.01	7.24 ± 0.03*, †
P_{CO_2} (mm Hg)	Room air	45.5 ± 3.0	47.7 ± 3.1	51.9 ± 4.8	61.0 ± 6.9
	Oxygen	51.0 ± 5.4	55.1 ± 4.3	56.6 ± 5.7	67.1 ± 6.2*, †
P_{O_2} (mm Hg)	Room air	30.8 ± 7.8	26.6 ± 7.3	19.8 ± 4.1	14.8 ± 2.8
	Oxygen	50.7 ± 10.4	38.3 ± 12.3	32.0 ± 7.1	18.9 ± 2.2*, †, ‡

Data are mean ± SD from six experiments.
*Significant effect of blood flow reduction.
†Significant effect of oxygen.
‡Significant interaction between blood flow reduction and oxygen.

Table IV. The effect of maternal oxygen administration on fetal oxygenation during graded reductions in uterine blood flow

Variable	*Condition*	*Baseline*	*Uterine blood flow reduction*		
			30%	*50%*	*70%*
Uterine blood flow (% of control)	Room air	100	73 ± 4	50 ± 8	30 ± 3
	Oxygen	100	71 ± 5	50 ± 7	31 ± 3*
Umbilical blood flow (ml/min/kg)	Room air	202 ± 14	205 ± 13	211 ± 20	219 ± 29
	Oxygen	201 ± 12	207 ± 19	212 ± 24	233 ± 26*
Umbilical vein oxygen (ml oxygen/dl)	Room air	11.4 ± 0.9	10.1 ± 1.1	7.3 ± 1.5	3.8 ± 1.2
	Oxygen	13.7 ± 2.2	12.1 ± 1.4	10.3 ± 2.1	5.4 ± 2.1*, †
Descending aorta oxygen (ml oxygen/dl)	Room air	8.0 ± 1.0	6.7 ± 1.5	4.5 ± 1.3	2.3 ± 0.8
	Oyxgen	10.2 ± 1.6	8.6 ± 1.2	7.2 ± 1.7	3.3 ± 1.3*, †
Oxygen delivery (ml oxygen/min/kg)	Room air	23.0 ± 2.0	20.8 ± 2.2	15.4 ± 3.0	8.3 ± 2.4
	Oxygen	27.4 ± 3.7	25.0 ± 3.0	21.9 ± 4.4	12.3 ± 3.6*, †
Increase in oxygen delivery (ml oxygen/min/kg)		4.4 ± 3.9	4.2 ± 2.1	6.5 ± 2.6	4.0 ± 2.6
Oxygen consumption (ml oxygen/min/kg)	Room air	6.8 ± 1.6	7.0 ± 1.4	5.9 ± 1.2	3.3 ± 0.8
	Oxygen	6.9 ± 1.3	7.3 ± 1.9	6.6 ± 1.7	4.7 ± 1.6*, ‡
Increase in oxygen consumption (ml oxygen/min/kg)		0.1 ± 0.7	0.3 ± 1.0	0.7 ± 1.2	1.4 ± 1.3§

Data are mean ± SD from six experiments.
*Significant effect of blood flow reduction.
†Significant effect of oxygen.
‡Significant interaction between blood flow reduction and oxygen.
§$p < 0.05$ compared with baseline.

increase compared with room air (Tables II, IV). Fetal oxygen consumption remained unchanged.

Graded reductions of umbilical blood flow resulted in a significant increase in fetal hemoglobin concentration (Table I). Fetal descending aortic oxygen saturation decreased significantly, whereas umbilical venous oxygen saturation remained unchanged. Fetal oxygen delivery decreased in proportion to the grade of umbilical blood flow reduction (Table II). With a reduction of fetal oxygen delivery below 12 ml/min/kg, fetal oxygen consumption began to decrease.

Maternal oxygen administration during graded reductions of umbilical blood flow decreased umbilical venous pH and increased umbilical venous P_{CO_2} significantly (Table I), probably due to maternal hypercapnia. Umbilical venous P_{CO_2} and oxygen saturation

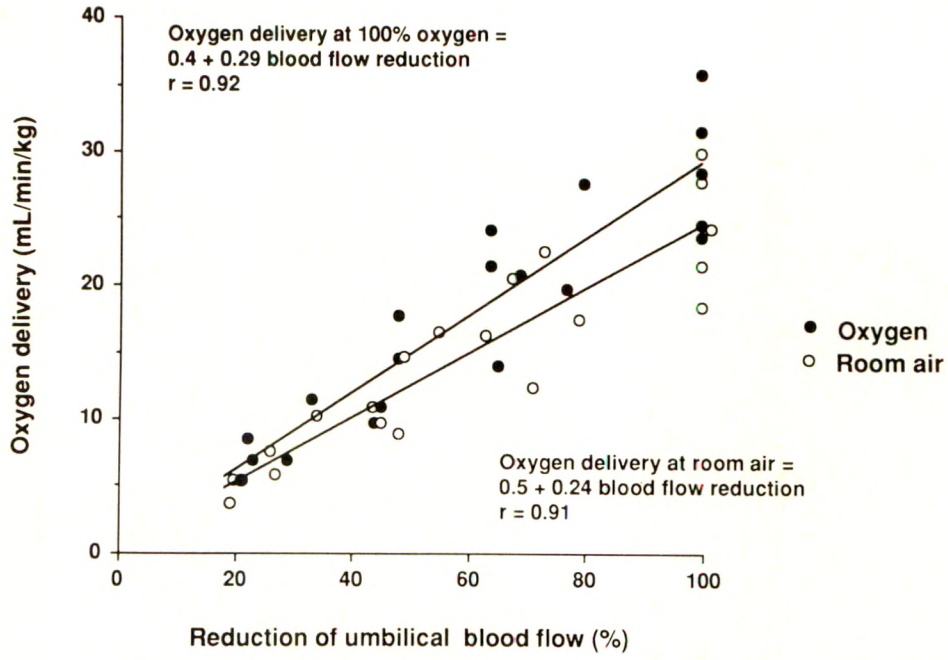


Fig. 1. Effect of maternal oxygen administration on fetal oxygen delivery during graded reductions of umbilical blood flow.

increased significantly. As a result, umbilical venous oxygen content increased to about 15.5 ml/min/kg at all grades of blood flow reduction (Table II). Thus maternal oxygen administration increased fetal oxygen delivery by 20% compared with the corresponding room air values. There was a significant interaction between the effect of maternal oxygen administration and blood flow reduction; although oxygen administration increased fetal oxygen delivery by 4.4 ml/min/kg at baseline, increases were 3.4, 2.7, and 1.3 ml/min/kg with a 30%, 50%, and 75% reduction of umbilical blood flow. Thus the beneficial effect of maternal oxygen administration on fetal oxygen delivery diminished with the severity of blood flow reduction. Maternal oxygen administration did not increase fetal oxygen consumption significantly.

Fig. 1 illustrates the relationship of fetal oxygen delivery to umbilical blood flow. In both room air– and oxygen-treated fetuses oxygen delivery was linearly related to umbilical blood flow. Fetal oxygen delivery was significantly greater in fetuses treated with oxygen. However, this increase was related to umbilical blood flow and diminished with the severity of blood flow reduction.

Graded reductions of uterine blood flow resulted in a significant increase in fetal hemoglobin concentration (Table III). Fetal arterial and umbilical venous oxygen saturation decreased significantly; umbilical blood flow increased by a modest degree. Fetal oxygen delivery decreased when uterine blood flow fell below 60% of baseline values (Table IV). With a reduction of fetal oxygen delivery below 15 ml/min/kg, fetal oxygen consumption began to decrease.

Maternal oxygen administration during reductions of uterine blood flow decreased umbilical venous pH and increased umbilical venous P_{CO_2} significantly (Table III), indicating maternal hypercapnia. Umbilical venous P_{CO_2} and oxygen saturation increased significantly. As a result, maternal oxygen administration increased umbilical venous oxygen content by about 2 ml/dl at all grades of blood flow reduction (Table IV). Because umbilical blood flow remained unchanged, maternal oxygen administration increased fetal oxygen delivery by about 4 ml/min/kg at all grades of uterine blood flow reduction. There was no significant interaction between the effect of maternal oxygen administration and blood flow reduction; thus the beneficial effect of maternal oxygen administration on fetal oxygen delivery was about the same at baseline and at 30%, 50%, and 70% reductions of uterine blood flow. Maternal oxygen administration increased fetal oxygen consumption significantly by 1.4 ± 1.3 ml/min/kg when uterine blood flow was reduced to 30% at baseline.

Fig. 2 illustrates the relationship of fetal oxygen delivery to uterine blood flow reduction. In room air– and oxygen-treated fetuses, oxygen delivery was logarithmically related to uterine blood flow. Fetal oxygen delivery was significantly greater in fetuses treated with oxygen. The increase in fetal oxygen delivery by maternal oxygen administration was not related to the grade of uterine blood flow reduction, even when

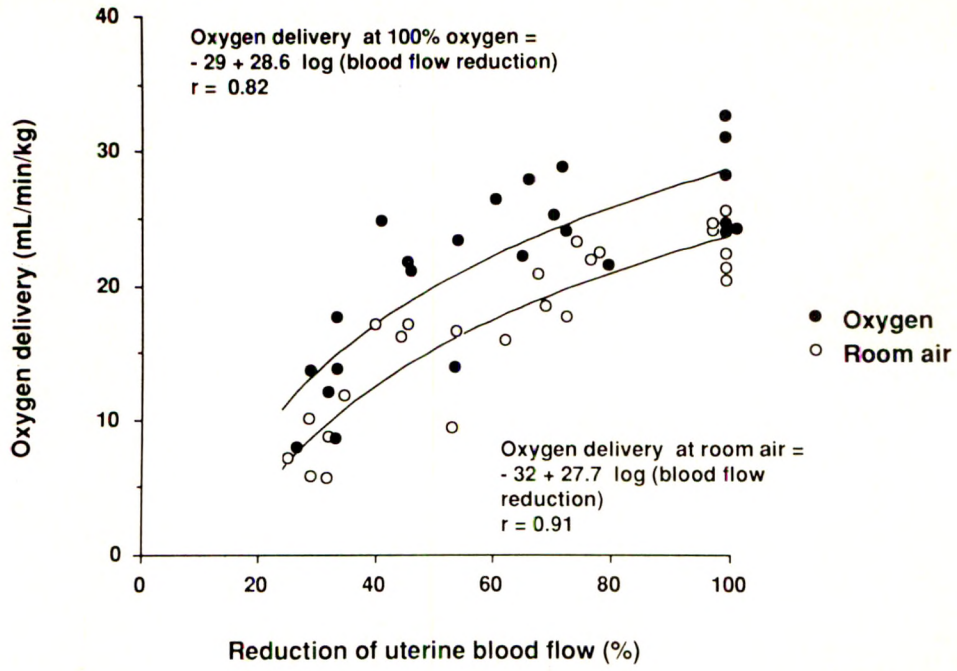


Fig. 2. Effect of maternal oxygen administration on fetal oxygen delivery during graded reductions of uterine blood flow.

uterine blood flow was reduced to 30% of baseline values.

Comment

The aim of the present study was to assess the effect of maternal oxygen administration on fetal blood gases, oxygen delivery, and oxygen consumption during graded reductions of uterine blood flow. The effect of maternal oxygen administration during reductions of uterine blood flow was also compared with that during reductions of umbilical blood flow of similar degree.

To reduce uterine blood flow, we used a snare placed around the common uterine artery. The effect of constricting the snare was monitored with a flow probe placed around the main uterine artery supplying the pregnant horn. Although the method is easy to use, certain limitations must be kept in mind. First, the uteroplacental circulation receives some blood supply from the ovarian arteries, which were not constricted by the snare, and second, although constriction of the snare should produce equal blood flow reduction in both uterine arteries, this may not always be true.[7] Because of these limitations, changes in uteroplacental blood flow may not have been accurately reflected by measurement of flow in one uterine artery.

Our results on the effect of reducing umbilical blood flow are in close agreement with previous studies.[8, 9] Umbilical venous oxygen content did not decrease during reduction of umbilical blood flow; thus fetal oxygen delivery was linearly related to umbilical blood flow. Fetal oxygen consumption was maintained with reduction of umbilical blood flow to about 50% of baseline values; further reductions were associated with a progressive fall in oxygen consumption.

The effect of maternal oxygen administration on fetal oxygenation during reduction of umbilical blood flow has been studied previously.[4] From these studies it was concluded that maternal oxygen administration increases oxygen delivery to the fetus during times of reduced umbilical perfusion. However, because umbilical venous oxygen content does not decrease and may actually increase during reduction of umbilical blood flow,[9] only a small amount of oxygen can be added by maternal oxygen administration. Under these conditions umbilical blood flow is the main determinant of fetal oxygen delivery; the beneficial effect of maternal oxygen administration diminishes with the severity of blood flow reduction. Thus maternal oxygen administration has only a modest effect on fetal oxygen delivery when umbilical blood flow falls below 50% of baseline values.

The effect of uterine blood flow reduction on fetal oxygenation has been studied extensively.[10-12] In agreement with these studies our results demonstrate that the oxygen supply to the pregnant uterus exceeds the minimum necessary to maintain fetal oxygen delivery. Thus fetal oxygen delivery started to decrease only when uterine blood flow fell below 60% of baseline values.

A previous study investigated the effect of maternal

oxygen administration on fetal oxygenation in chronically hypoxemic, growth-retarded fetal sheep.[5] In this study maternal arterial oxygen content and umbilical blood flow were in the physiologic range of values. Thus it may be speculated that the underlying cause for chronic hypoxemia in these fetuses was a severe reduction in uterine blood flow. Maternal oxygen administration increased fetal oxygen delivery by about 5.5 ml/min/kg.[5] In our present study maternal oxygen administration has been shown to have a significant effect on fetal oxygen delivery, even with a short-term reduction in uterine blood flow to 30% of baseline.

During reduction of uterine blood flow umbilical venous oxygen saturation decreases significantly. Thus a substantial amount of oxygen can be added to umbilical venous blood. For example, when uterine blood flow was reduced to 30% of baseline, maternal oxygen administration increased umbilical venous oxygen content from 3.8 to 5.4 ml/dl. Because umbilical blood flow did not change, maternal oxygen administration increased fetal oxygen delivery from 8.3 to 12.3 ml/min/kg, representing a 50% increase.

In summary, we have demonstrated that maternal oxygen administration has a significant effect on fetal oxygenation during short-term reductions of uterine or umbilical blood flow. However, the beneficial effect of maternal oxygen administration is far greater during reductions of uterine blood flow than during reductions of umbilical blood flow. If the response of the human fetus to maternal oxygen administration is similar to that of the sheep fetus, maternal oxygen administration may be beneficial to the fetus during labor, especially when the fetal heart rate pattern is suggestive of reduced uterine blood flow.

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Convulsions in pregnancy in related gorillas

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Two related gorillas, the second the granddaughter of the first, had pregnancies complicated by convulsions and edema. They may have suffered from eclampsia, indicating that this is not an exclusively human disease and that in gorillas, as in humans, there is a familial factor. (Am J Obstet Gynecol 1992;167:240-1.)

Key words: Eclampsia, gorillas, pregnancy

It is generally considered that *Homo sapiens* is the only species affected by the pregnancy-specific diseases pre-eclampsia and eclampsia, although single cases of possible eclampsia[1] and preeclampsia have been reported in captive lowland gorillas. We wish to draw researchers' attention to another published case of probable eclampsia affecting the maternal grandmother in the case reported by Baird.[1]

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Case report

The case in question was mentioned by George Schaller in his classic book, *The Mountain Gorilla,*[2] but there is no published reference to the fact that the gorillas were related. He reported that the first successful gorilla pregnancy in captivity at the Columbus Zoo, Columbus, Ohio, in 1956 was complicated by con-

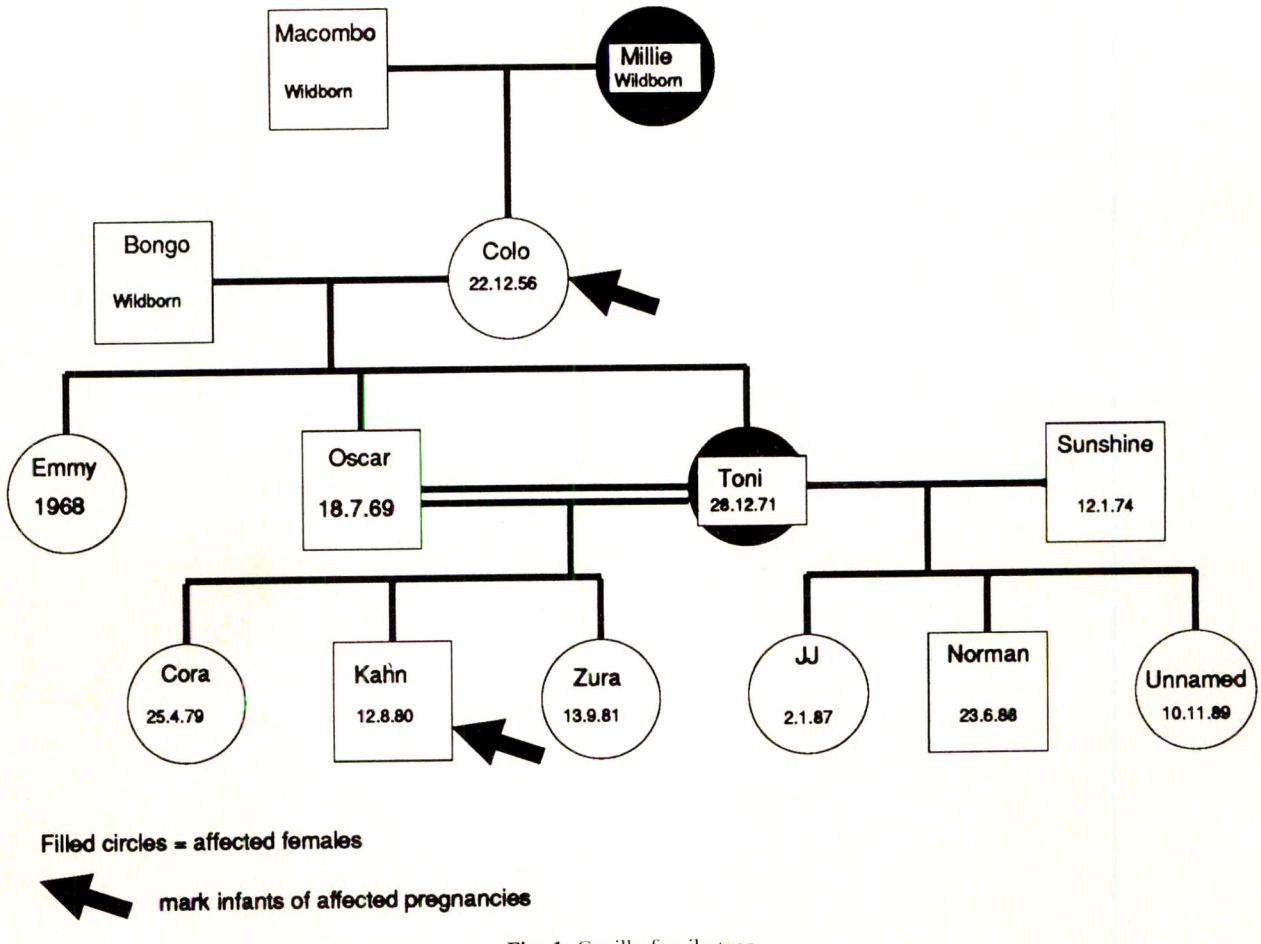


Fig. 1. Gorilla family tree.

vulsions and edema. The mother, Millie, delivered a female infant named Colo, which survived, but no further details were given. Colo had three offspring, a son and two daughters. One of the daughters, Toni, had three pregnancies by her brother Oscar. The first and third pregnancies were normal and resulted in the delivery of healthy daughters, but the second was complicated by convulsions and edema.[1] The blood pressure was not recorded, urine was not tested for protein, and the gestation was uncertain. The mother was treated with magnesium sulfate, and the pregnancy continued for 2 weeks after the convulsions. The male baby was alive at birth but died of salmonella sepsis at 18 days. The placenta was infarcted. Toni subsequently had three further pregnancies without complication and at the time of writing is pregnant again. The family tree is shown in Fig. 1. Both these cases are suggestive of eclampsia. Neither mother had convulsions before and after the affected pregnancies, and Millie went on to live another 20 years after the delivery of Colo, although she never had another pregnancy. There is no other disease with which they are likely to have been affected.

Comment

These two cases have prompted us to set up a data base of gorilla births in captivity. This is not yet complete, but there may have been another case of pre-eclampsia. That pregnancy was complicated by proteinuria (2+ to 3+) (specimen collected from the cage floor) and resulted in the stillbirth of a 2200 gm female fetus. There was evidence of abruptio placentae and infarction. This was the third of five pregnancies, paternity having changed after the fourth only. All the others resulted in live births. We have details of >200 gorilla births in captivity worldwide. A rate of two cases of eclampsia is similar to that in humans before the availability of widespread antenatal care, where rates of one to two per thousand pregnancies have been reported. We would be interested to hear of any other cases that may have gone unreported.

It would be surprising if preeclampsia were an exclusively human disease. Gorillas and chimpanzees are the primates most closely related to man, not only an atomically and behaviorally but also at the molecular level. In common with man both species have a hemochorial placenta and a comparable gestation period. Orangutans have a slightly shorter gestation, and although we can find no evidence of eclampsia occurring in an orangutan, one at the San Diego Zoo with a pregnancy complicated by proteinuria delivered a stillborn infant. The occurrence of eclampsia in related gorillas may support a genetic etiology. In humans some workers have demonstrated inheritance patterns compatible with mendelian inheritance, although our twin studies have recently provided conflicting data.[3]

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Messenger ribonucleic acid for transforming growth factor-α, but not for epidermal growth factor, is expressed in fetal and neonatal mouse brain

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OBJECTIVE: Little is known concerning the presence of transforming growth factor-α in fetal and neonatal tissues. Our objective was to analyze messenger ribonucleic acid expression for transforming growth factor-α and epidermal growth factor, the structural and biochemical analog of transforming growth factor-α in fetal and neonatal murine brain.

STUDY DESIGN: Messenger ribonucleic acid was prepared from whole brains of mice from these developmental stages: embryonic days 14, 15, and 17, postnatal days 0, 4, 10, and adult. Polymerase chain reaction was performed on the complementary deoxyribonucleic acid obtained from reverse transcription of messenger ribonucleic acid with transforming growth factor-α and epidermal growth factor primers. In addition, ribonuclease protection assay was used to identify transforming growth factor-α transcripts.

RESULTS: We found messenger ribonucleic acid encoding transforming growth factor-α in all stages of development used. Epidermal growth factor messenger ribonucleic acid was not found in any stage. Ribonuclease protection assay confirmed transforming growth factor-α transcripts in these tissues.

CONCLUSION: Transforming growth factor-α may play an autocrine or paracrine role in the differentiation or maintenance of murine fetal and neonatal brains. (Am J Obstet Gynecol 1992;167:242-5.)

Key words: Transforming growth factor-α, epidermal growth factor, brain development, growth factors

Transforming growth factor-α (TGF-α) is a 50-amino-acid peptide that causes a reversible transformation of phenotype in normal rat kidney cells.[1] It is a biochemical and structural homolog to epidermal growth factor (EGF) in that it has 30% to 40% amino acid identity and similar three-dimensional characteristics. Both growth factors act through the same 170 kd tyrosine kinase receptor, the EGF receptor, for signal transduction. The two growth factors have been implicated as autocrine or paracrine regulators of cellular function.

In situ hybridization analysis has revealed the presence of transforming growth factor-α messenger ribonucleic acid (mRNA) in adult mouse brain.[2] In situ hybridizations performed with whole fetal mice show TGF-α mRNA in the fetal otic vesicle and oral cavity, among other places, but no TGF-α mRNA was found after embryonic day 10.[3] Kudlow et al.[4] demonstrated that fetal rat hypothalamus and brain stem contained TGF-α mRNA but did not specify the developmental stage of the fetus studied.

Reverse transcription of mRNA into complementary deoxyribonucleic acid (cDNA) and then amplification of the cDNA by polymerase chain reaction has allowed the analysis of small quantities of mRNA. This method has been used to demonstrate that mRNA encoding TGF-α, but not EGF, is expressed in preimplantation mouse embryos.[5] We used this method and ribonuclease protection assay to study the developmental expression of TGF-α and EGF mRNA in fetal and neonatal murine brain. The presence of TGF-α mRNA would suggest a possible autocrine or paracrine role for this growth factor in brain development.

Material and methods

mRNA preparation. C57BL/6j mice were obtained from the breeding colony of the Children's Hospital Mental Retardation Research Center. The care and use of the animals were approved by the Children's Hospital animal care committee. Whole brains were isolated from the following developmental stages: embryonic

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days 14, 15, and 17, postnatal days 0, 4, and 10, and a 60-day-old adult. RNA was isolated with the guanidine isothiocyanate method, followed by centrifugation through cesium chloride.[6, 7]

Reverse transcription. The mRNA from the murine brains was used as template for the synthesis of cDNA by the following method[5]: Two micrograms of oligodeoxythymidylic acid (12 to 18) (Pharmacia, Piscataway, N.J.) were used as primer for each reverse transcription reaction. Five hundred nanograms of RNA and oligodeoxythymidylic acid in a final volume of 20 μl was heated to 68° C for 2 minutes and then cooled to 25° C for 2 minutes. 5× BRL reverse transcription buffer (BRL, Gaithersburg, Md.), 2 μl RNAsin (Promega, Madison, Wis.), 16 μl 2.5 mmol/L deoxynucleotides, and 400 U Moloney murine leukemia virus reverse transcriptase (BRL) were added to the mRNA and oligodeoxythymidylic acid mixture in a final volume of 50 μl. This mixture was incubated at 42° C for 45 minutes. An additional 400 U Moloney murine leukemia virus reverse transcriptase was added, and the mixture was incubated at 42° C for a further 45 minutes. The incubation was terminated by heating to 68° C for 10 minutes and then stored at −20° C until used for polymerase chain reaction.

Polymerase chain reaction. TGF-α primers used were 5′ACCTGCAGGTTTTTGGTGCAG3′ and 5′GCAGACGAGGGCACGGCACCA3′.[5] The two TGF-α primers encompass a 239 bp nucleic acid region encoding bases 58 to 78 (5′-oligonucleotide) and bases 297 to 277 (3′-oligonucleotide), respectively, and with DNA sequence having a unique restriction endonuclease site for *Sph* I such that fragments of 159 and 80 bp are generated after cleavage. EGF primers used were from the mouse cDNA sequence and were 5′AATAGTTATCCAGGATGCCCA3′ (bases 3238 to 3259) and 5′TCGTAGGTCTCGAGTCTGACATCG3′ (bases 3381 to 3357).[8] The primers encompass the sequence encoding the first 48-amino-acid residues of the mature EGF peptide.

Polymerase chain reaction DNA amplification used 10 μl of the reverse transcribed cDNA.[5] A Perkin-Elmer/Cetus GeneAmp (Perkin-Elmer/Cetus, Norwalk, Conn.) kit was used according to manufacturer's instructions. Thirty cycles of the following thermocycling conditions were used: 94° C for 20 seconds, 55° C for 20 seconds, and 72° C for 30 seconds. mRNA from whole adult mouse ovary was used as positive control for TGF-α and adult male mouse salivary mRNA was a positive control for EGF for reverse transcription polymerase chain reaction. Yeast transfer RNA was used as negative control.

Analysis of polymerase chain reaction products. The amplified DNA fragments were analyzed without further purification by electrophoresis on 3% NuSieve and 1% SeaKem (FMC, Rockland, Me.) agarose gels. Before digestion with the restriction endonuclease *Sph* I, the polymerase chain reaction–amplified DNA fragments were chloroform extracted, ethanol precipitated, and resuspended in sterile water.

Ribonuclease protection assay. A TGF-α 239 bp polymerase chain reaction fragment was cloned into a Bluescript phagemid vector by the method of Marchuk et al.[9] This TGF-α clone was sequenced to confirm its identity and orientation. After that, in vitro transcription of TGF-α was performed (SP6/T7 Transcription Kit, Boehringer/Mannheim, Indianapolis), with α-^{32}phosphorus cytidine triphosphate (Amersham, Arlington Heights, Ill.) as the label, and the radiolabeled TGF-α RNA probe was purified with a 5% acrylamide gel. This purified TGF-α probe was then hybridized overnight at 45° C to 10 μg of murine brain RNA (RPA II Kit, Ambion, Austin, Tex.). The hybridized RNA was digested with ribonuclease A and T1 for 30 minutes at 37° C, then was subjected to electrophoresis in a 5% acrylamide gel. After that, the gel was exposed to Kodak XAR-5 film (Eastman Kodak Co., Rochester, N.Y.) at −70° C with an intensifying screen (Dupont Cronex Lightning Plus, Dupont Instruments, Wilmington, Del.) for 2 days. The positive control used was adult rodent ovary, which is known to contain TGF-α mRNA. The negative control was yeast RNA.

Northern blotting. RNA was fractionated on 2.2 mol/L formaldehyde and 1.2% agarose surface tension gel and transferred to a charged nylon membrane (Nytran, Schleicher, and Schuell, Keene, N.H.). The blots were hybridized in 50% formamide, 5× saline solution–sodium citrate buffer at 48° C overnight. A 925 bp fragment from the TGF-α probe phTGF1-10-925 (gift of G.I. Bell) was labeled (specific activity of 1×10^9 disintegrations/min/μg) with a random hexanucleotide priming kit (Boehringer-Mannheim, Indianapolis) and α-^{32}P deoxycytidine triphosphate (Amersham). After hybridization and washing, the filters were exposed to Kodak XAR-5 film at −70° C with an intensifying screen.

Results

With reverse transcription and then polymerase chain reaction, amplification products specific for TGF-α were detected in the brains at the following stages of development: embryonic days 14, 15, and 17, postnatal days 0, 4, and 10, and adult brain (Fig. 1). Restriction endonuclease digestion by *Sph* I of the polymerase chain reaction–amplified DNA revealed appropriate hydrolysis of the 239 bp TGF-α sequences into fragments of 159 and 80 bp (Fig. 1). With the cDNA from the same reverse transcription reaction, polymerase chain reaction amplification was performed with primers for EGF. No polymerase chain reaction–amplified

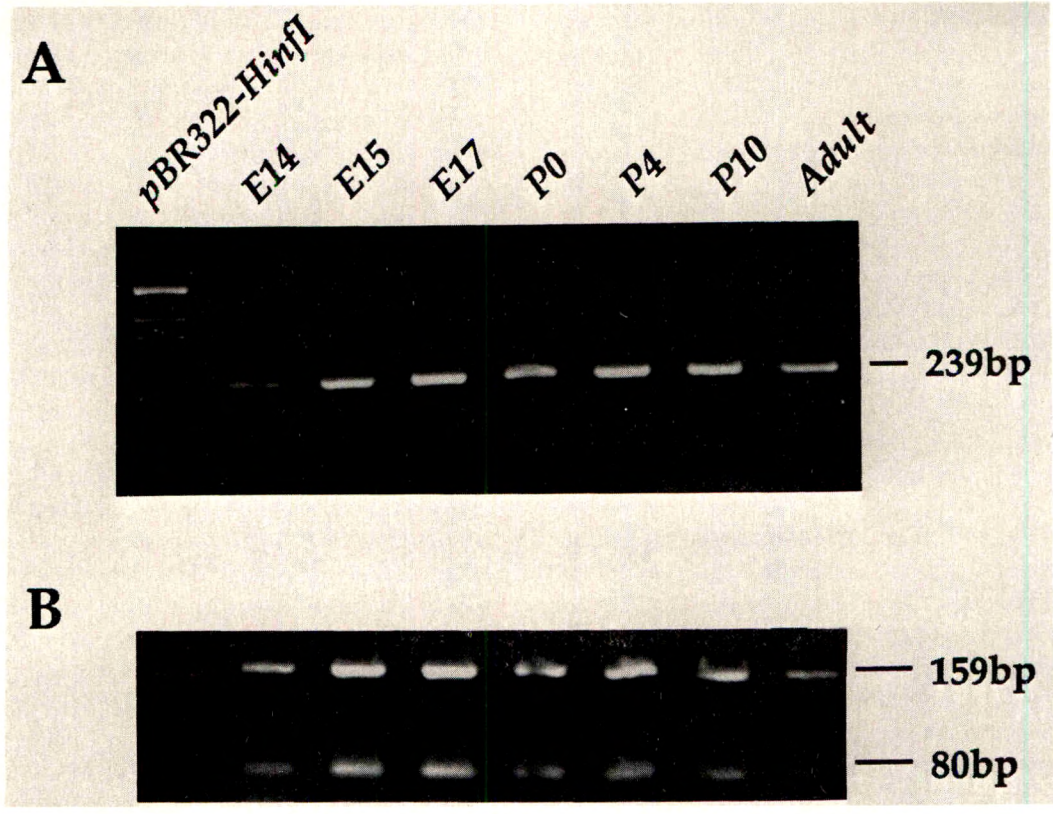


Fig. 1. A, Reverse transcription followed by polymerase chain reaction with primers for TGF-α of murine fetal (embryonic days *E14, E15,* and *E17*) and neonatal (postnatal days *P0, P4,* and *P10*) brain mRNA results in 239 bp DNA in all developmental stages studied. **B,** Digestion of the reverse transcribed polymerase chain reaction 239 bp DNA fragments with *Sph* 1 results in the predicted fragments of 159 and 80 bp, thereby confirming that polymerase chain reaction–amplified fragments encode TGF-α.

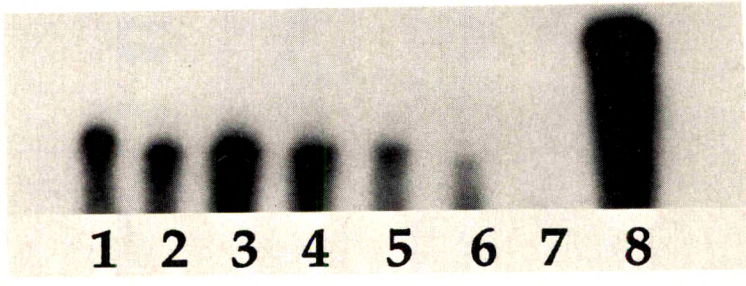


Fig. 2. Ribonuclease protection assay with 10 μg to total RNA from whole mouse brain hybridized with 239 bp TGF-α RNA probe. *Lanes 1 to 5* represent the following developmental stages: embryonic days 15 and 17 and postnatal days 0, 4, and 10. *Lane 6*, adult rodent ovary. *Lane 7*, Yeast negative control, *Lane 8*, Undigested radiolabeled TGF-α RNA probe.

DNA encoding EGF was observed in the brain developmental series. Mouse salivary gland mRNA, which is a positive control for EGF, resulted in the expected polymerase chain reaction amplification product.

Ribonuclease protection assay was used as a second method to confirm the expression of TGF-α in fetal and neonatal brain (Fig. 2). Ten micrograms of total RNA from embryonic days 15 and 17 and postnatal days 0, 4, and 10 murine brain was used for analysis and all developmental stages showed the presence of TGF-α transcripts. Northern analysis with up to 20 μg RNA was used as another method to confirm the presence of TGF-α and revealed faint positive signals at the expected 4.5 kb TGF-α mRNA location.

Comment

We conclude that fetal and neonatal expression of TGF-α, but not EGF, mRNA occurs in both the devel-

oping and mature mouse brain. Ribonuclease protection analysis also shows that TGF-α expression occurs in fetal and neonatal murine brain.

Rappolee et al.[5] detected mRNA for TGF-α, but not EGF, in preimplantation murine embryos with reverse transcription followed by polymerase chain reaction amplification of cDNA. Paria and Dey[10] found that addition of TGF-α or EGF to the culture medium of mouse blastocytes improved embryo development. They also showed that EGF receptors are present in eight-cell embryos. These data suggest that TGF-α or EGF may have an important role in the development of preimplantation and periimplantation mouse embryos.

Previous investigators have reported the presence of TGF-α in fetal rodent brains but have not reported expression of TGF-α in a developmental series. Brown et al.[11] found evidence for TGF-α by radioimmunoassay in late gestational fetal rat brains (embryonic day 20), with peak levels observed in late gestation. Ojeda et al.[12] reported that mouse embryonic day 18 fetal hypothalamus had TGF-α mRNA, by Northern analysis, but not EGF mRNA. They also reported that higher levels of TGF-α mRNA was observed on embryonic day 18 than postnatally. Kudlow et al.[4] showed that fetal rat hypothalamus and brain stem contained TGF-α mRNA but did not specify the developmental stage of the fetus studied. We used reverse transcription followed by polymerase chain reaction, a much more sensitive method of detection of mRNA encoding for TGF-α, to study the developmental expression of TGF-α and found that TGF-α mRNA was expressed in all stages studied, starting from embryonic day 14. Our evidence confirms that TGF-α mRNA is expressed in fetal mouse brain.

Cell culture and in vivo studies indicate that TGF-α or EGF may have a physiologic function in brain tissues. Cultures of fetal rat telencephalon with EGF cause a stimulation of lectin production, which in turn may be related to neuronal aggregation.[13] TGF-α and EGF increased gonadotropin-releasing hormone release from juvenile rodent female median eminances in a dose-related fashion.[12] The mRNA for both TGF-α and its receptor, the EGF receptor, are present in the bovine pituitary.[14] In vivo, Polk et al.[15] infused EGF in fetal sheep and found resultant increased corticotropin levels, suggesting that EGF or TGF-α may have pituitary functions. Thus TGF-α and its receptor may have physiologic roles in the regulation of brain and pituitary function. The most likely reason for our finding that TGF-α, but not EGF, mRNA is present in the fetal and neonatal tissues is that TGF-α is the physiologic factor that acts as the autocrine or paracrine ligand for the EGF receptor in the mouse brain. Furthermore, our data that TGF-α mRNA is expressed both in fetal and in neonatal brains suggest that TGF-α may be important both in utero and post partum.

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Cardiac electromechanical dissociation in the hypoxic fetal sheep may have clinical implications

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OBJECTIVES: Our purpose was to determine the sequence of fetal cardiac electrical and mechanical events associated with acute hypoxic acidemia and to correlate those events with terminal fetal heart rate patterns preceding fetal death.

STUDY DESIGN: Eight acutely catheterized fetal sheep were rendered hypoxic by placental embolization with microspheres until fetal death occurred. The fetal electrocardiogram, Doppler cardiogram, left ventricular and aortic pressures, and fetal heart rate were continuously recorded.

RESULTS: All eight fetuses showed a terminal bradycardia consisting of two phases: an initial phase of falling ventricular pressures, culminating in mechanical asystole, and a subsequent phase after asystole during which the electrocardiographic signal persisted for an average duration of 15.2 ± 8.7 minutes (range 3.1 to 32.4) and triggered a Hewlett-Packard 8040A monitor to show a heart rate pattern.

CONCLUSIONS: The phenomenon of cardiac electromechanical dissociation may be occurring during similar terminal bradycardias that are observed in human labor and explains the delivery of a fresh stillbirth in spite of the recording of an electrocardiographic signal from a scalp electrode. (Am J Obstet Gynecol 1992;167:246-9.)

Key words: Fetal death, fetal electrocardiogram, electromechanical dissociation, fetal monitoring, fetal sheep

Electromechanial dissociation is the persistence of electrocardiographic complexes in the absence of effective cardiac output. In adults it is usually due to a significant reduction in preload, as in exsanguinating hemorrhage or pericardial tamponade, or to a marked increase in afterload, such as in massive pulmonary embolism. Electromechanical dissociation may also reflect severe dysfunction of the cardiac pump.[1] Myocardial ischemia has been implicated in the development of electromechanical dissociation, and it is the most frequent cause of unsuccessful resuscitation in critically ill adults, accounting for up to 68% of sudden death after myocardial infarction.[2]

Although many obstetricians will have had the experience of delivering a fresh stillbirth after a sustained bradycardia, to our knowledge the cardiac electromechanical events underlying such terminal fetal heart rate (FHR) patterns have not been described. We now report the observation of electromechanical dissociation in hypoxemic and acidemic fetal sheep and discuss the clinical implications of this finding for electronic monitoring of the human fetus in labor.

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6/1/36690

Material and methods

Surgical procedure. Surgery was performed on eight pregnant ewes, each with a singleton gestation between 132 and 134 days. Anesthesia was induced with thiopental sodium infused intravenously and was maintained by positive pressure ventilation with isoflurane, 1.25% to 2%, and oxygen. The ewes were mildly hyperventilated (tidal volume 700 ml, respiratory rate 20 breaths/min) to prevent carbon dioxide accumulation. The fetal head, forelimbs, and chest were withdrawn through a uterine incision. Catheter-tip pressure transducers (2F, Millar, Houston) were introduced into the left ventricle and the aorta just above the aortic valve through a carotid artery (locations confirmed at necropsy). Three electrocardiogram (ECG) electrodes were sutured to the fetal chest. The fetus was replaced and the uterine incision was closed. The hind part of the fetus was then withdrawn through a separate uterine incision, and a fluid-filled catheter was introduced into the aorta through a femoral artery and used to obtain blood samples and inject microspheres for placental embolization. The hind part of the fetus was then replaced, and the second uterine incision was closed. The catheters and ECG leads were exteriorized through the abdominal incision of the ewe, which was

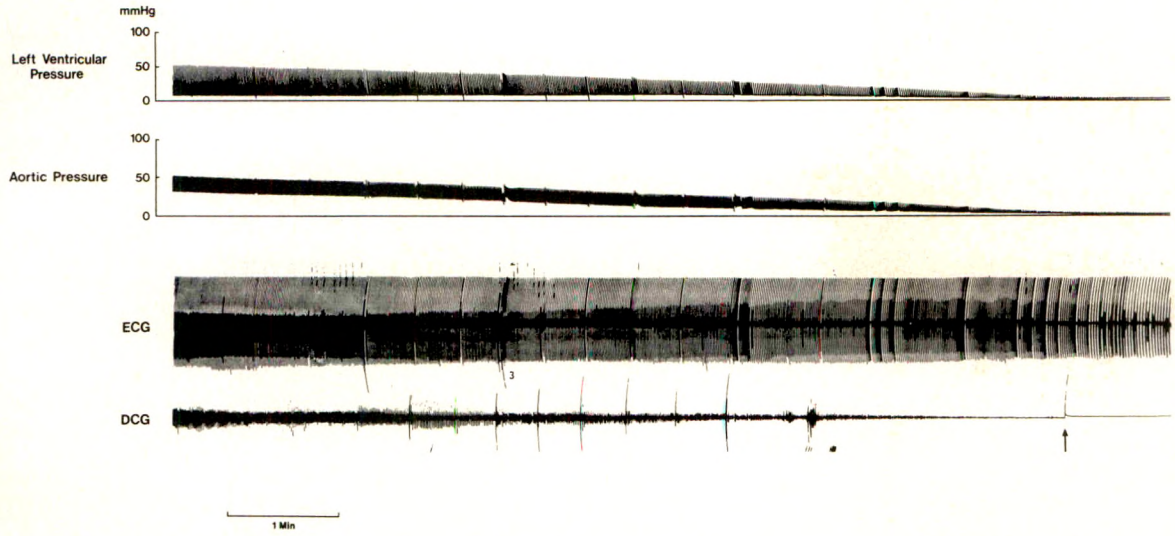


Fig. 1. Cardiac electrical and mechanical waveforms at onset of electromechanical dissociation. *DCG*, Doppler cardiogram. Note that ECG signal continues beyond point of mechanical asystole, which is indicated by *arrow*.

held closed with clamps. A vascular catheter was placed in the femoral artery of the ewe to monitor maternal blood pressure and blood gases.

Experimental procedure. The ECG leads were connected to an FHR monitor (Hewlett Packard 8040A, Hewlett Packard Co., Palo Alto, Calif.), which also supplied a Doppler signal when the transducer was placed on the maternal abdomen and directed toward the fetal heart. Fetal left ventricular pressure, aortic pressure, ECG, Doppler cardiogram, mean arterial pressure, and heart rate were monitored continuously on a polygraph (Grass Instruments, Quincy, Mass.). Fetal arterial blood samples, 0.5 ml per sample to a total not exceeding 5 ml, were obtained at intervals throughout the experiments and were immediately analyzed for blood gas tensions and pH with a blood gas analyzer (model 170, Corning Medical, Medfield, Mass.). Fetal blood gas tensions were temperature corrected to 39° C.

Fetal hypoxemia was induced by embolizing the placenta with injections of 5×10^5 50 μm polyethylene microspheres (3M, St. Paul, Minn.),[3] repeated every 15 minutes until fetal death occurred.

The study was approved by the hospital animal care committee following the guidelines of the Canadian Council for Animal Care.

Results

In all eight fetuses electromechanical dissociation was demonstrated by the persistence of cardiac electrical activity after mechanical asystole. The maternal arterial blood gas tensions remained relatively constant throughout the experiments with a pH of 7.47 ± 0.09 (mean ± 1 SD), P_{CO_2} 32.5 ± 4.0 mm Hg, and P_{O_2} 143.4 ± 17.6 mm Hg. FHR before embolization of the placenta was 141 ± 22 beats/min. There were no significant changes in the FHR before the onset of terminal bradycardia with the exception of one fetus that showed a sinusoidal pattern that lasted for 7 minutes. The mean number of microsphere boluses required to cause fetal death was six (range 5 to 8). Left ventricular systolic pressures were maintained at relatively stable levels of 54.0 ± 8.7 mm Hg, until 15.5 ± 10.4 minutes before the onset of electromechanical dissociation, when they started to decline simultaneously with the onset of a terminal bradycardia. Fetal arterial pH decreased from an initial value of 7.33 ± 0.07 at the start of the experiment to 7.01 ± 0.06 at the onset of terminal bradycardia. Mechanical asystole occurred at a fetal pH of 6.90 ± 0.11 and was demonstrated by the cessation of ventricular and aortic pressure waveforms, at which time the Doppler signal also ceased (Fig. 1). Fetal arterial P_{O_2} and P_{CO_2} at the onset of bradycardia were 11.6 and 62.4 mm Hg, respectively. At the time of onset of electromechanical dissociation, their values were 8.9 and 71.6 mm Hg, respectively. The duration of electromechanical dissociation was 15.2 ± 8.7 minutes (range 3.1 to 32.4), during which time regular supraventricular ECG complexes persisted with a mean rate at the onset of electromechanical dissociation of 91.3 ± 6.5 beats/min. This electrical activity was sufficient to trigger the HP 8040A FHR monitor to show an FHR pattern that gradually declined to a rate of between 40 and 50 beats/min (Fig. 2). In five cases the episode of electromechanical dissociation ended with electrical asystole and in three cases with ventricular fibrillation. The possibility that the mother was the source of the ECG signal was excluded by a comparison of the fetal and maternal heart rates.

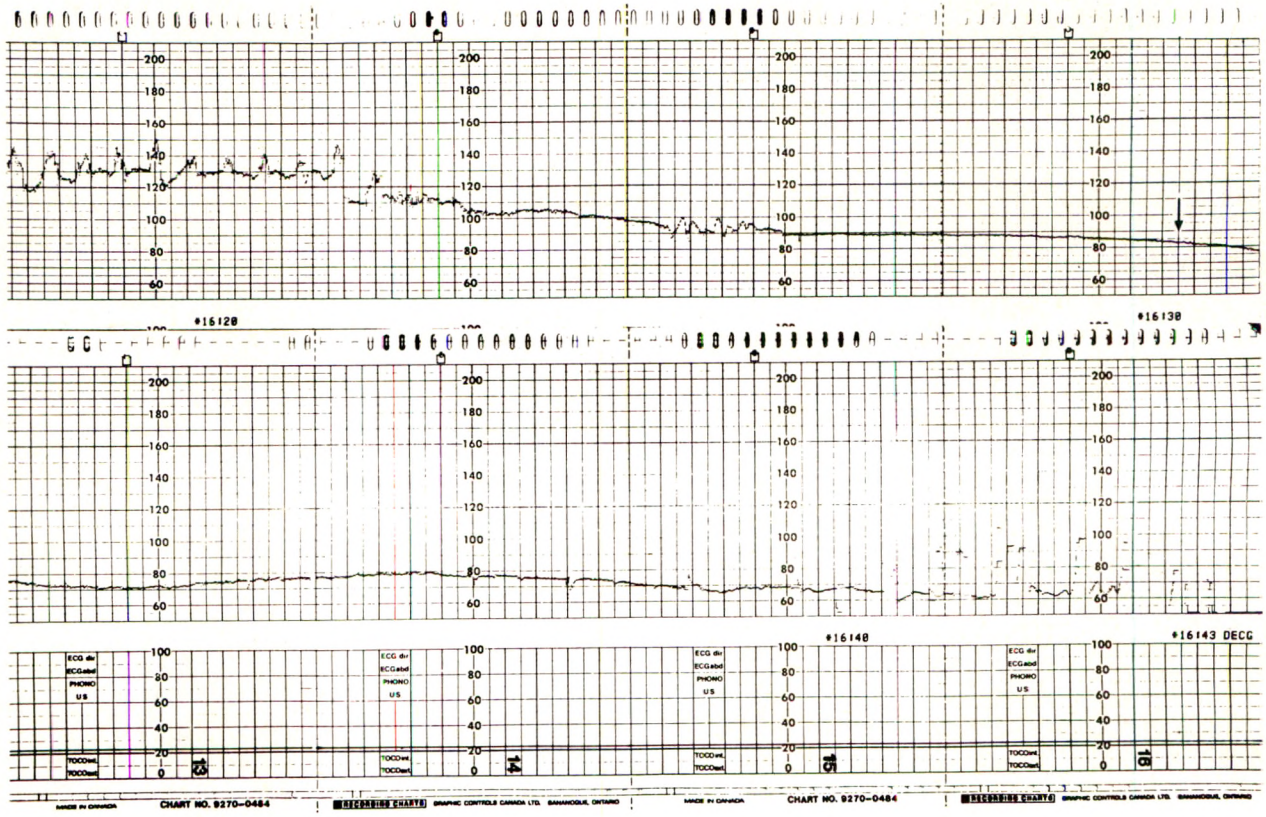


Fig. 2. Heart rate tracing showing a terminal bradycardia, from same fetal sheep as in Fig. 1. Heart rates <60 beats/min, although detected by the HP 8040A monitor, are not displayed on trace. *Arrow*, Mechanical asystole (fetal death) and onset of electromechanical dissociation. Upper and lower segments of trace are continuous (paper speed 3 cm/min).

Comment

In this experimental model of fetal asphyxia, a bradycardia culminating in electromechanical dissociation invariably occurred as a terminal event. We have previously shown that embolization of the fetal placenta in sheep results in a gradually deepening hypoxic acidemia[3] that closely resembles the blood gas changes observed with acute uteroplacental insufficiency and have thus elected to use this model in the present study. Although human fetal hypoxic acidemia is often slowly progressive, it may be accelerated by labor or other acute insults, resulting in a rapid deterioration of fetal blood gases as created by our experimental model. In other studies fetal hypoxemia with or without acidemia was created by interventions such as the induction of maternal hypoxemia, occlusion of the uterine or umbilical circulations, or by the direct infusion of acid into the fetal circulation. Clinically, maternal hypoxemia is very uncommon as a cause for fetal asphyxia. Furthermore, fetal asphyxia is a gradually progressive condition, whereas acidemia is paralleled by tissue acidosis. The intravascular administration of acid is not accompanied by carbon dioxide accumulation, and the degree of tissue acidosis generated by this intervention, especially myocardial tissue acidosis affecting contractility, might not accurately reflect clinical asphyxia.

The gradual decline in heart rate and the duration of the observed period of electromechanical dissociation is in keeping with observations from other species. Experimental electromechanical dissociation in adult dogs followed ventricular fibrillation induced by electrical current and persisted for >20 minutes,[2] during which time the initial rhythms were sinus or junctional tachycardias with a ventricular rate >100 beats/min. As electromechanical dissociation continued, the heart rate slowed, and 18 of 29 dogs developed complete atrioventricular block. Attempts to restore effective mechanical systole by various pharmacologic means were all unsuccessful. Similarly, during human adult electromechanical dissociation[2] the ECG demonstrated a gradually declining heart rate and in none of 36 patients was resuscitation successful. The pathophysiology of electromechanical dissociation is unknown, but the failure of myocardial contractility suggests a depletion of high-energy phosphate bonds.[2]

The hypothesis that electromechanical dissociation

also occurs in human fetal death is supported by published examples of terminal FHR tracings. Cetrulo and Schifrin[4] report four cases ending in fresh stillbirth where there was a profound and sustained terminal bradycardia recorded by a scalp electrode. Similarly, Kates and Schifrin,[5] Cibils,[6] and Perkins[7] report agonal FHR patterns culminating in bradycardia and fetal death. The duration of profound bradycardia before delivery of the dead fetuses in these published examples is similar to the observed time course of bradycardia in our fetal sheep. Fetal cardiac electrical asystole lasting for a few seconds was described by Kates and Schifrin[5] and carried with it a grave prognosis when it was associated with underlying fetal asphyxia. This is different from the long-lasting mechanical asystole with persistence of cardiac electrical activity observed in our study. On the basis of our experimental findings, we suggest that terminal bradycardias in human fetuses consist of two phases. During the initial phase before the occurrence of mechanical asystole, heart sounds are still audible in spite of falling ventricular pressures. In the second phase, electromechanical dissociation occurs, and the abdominal Doppler signal is lost while the ECG signal continues to trigger the FHR monitor. It is therefore not surprising that, in spite of the persistence of an ECG signal, the examples cited resulted in the delivery of dead infants who could not be resuscitated.

The only noninvasive test that could unmask electromechanical dissociation would be the observation of cardiac standstill on real-time ultrasonography. Ultrasonography is also important in excluding the possibility of the recording of a maternal ECG relayed through a dead fetus.[8, 9] It is important, however, to differentiate between the absence of fetal cardiac activity as a terminal event complicating severe asphyxia, which is likely to result in poor outcome, from asystole complicating an acute insult such as abruptio placentae or umbilical cord prolapse, which may have a more favorable outcome if emergency measures are taken in time.

In conclusion, we have documented the cardiac electromechanical events associated with terminal fetal bradycardia and have demonstrated electromechanical dissociation as a terminal event in the hypoxic fetal sheep. Furthermore, we suggest its occurrence may be an underlying factor in human intrapartum stillbirth.

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SYMPOSIUM

Pathophysiology of Infertility Associated with Endometriosis

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Introductory remarks

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Despite all the research accumulated over the years regarding endometriosis, the cause, pathophysiology, natural course, and other aspects of this disease remain enigmatic. In particular, of the variety of clinical problems that affect patients who have endometriosis, the most perplexing problem for all gynecologists is the treatment of infertility associated with endometriosis. Although a close link between endometriosis and infertility has long been recognized, a direct cause-and-effect relationship between endometriosis and infertility has not yet been proved. Thus far many plausible mechanisms for endometriosis-associated infertility have been postulated. However, no single mechanism can explain the cause for infertility in every case. This may reflect the complexity of the pathophysiology of endometriosis, the multifactorial mechanisms involved in endometriosis-associated infertility, or both. Indeed, the clinical link between endometriosis and infertility does not always exist. For instance, some women who have severe endometriosis may also have normal reproductive performance. In contrast, we know of many patients who cannot conceive despite long-standing treatment for infertility. Many gynecologists may be inclined to use medical or surgical methods to treat infertile women who have endometriosis, without being certain of the efficacy of these treatments when all routine treatments for infertility have failed. However, it has not yet been established whether the treatment of endometriosis per se benefits patients who have impaired reproductive performance. The answer to this question must wait for the elucidation of the mechanisms involved in endometriosis-associated infertility.

With this in mind, we held a conference on the "Pathophysiology of infertility associated with endometriosis" in Hakone, Japan, on September 6, 1991. At the conference we attempted to address this issue from various standpoints and placed special emphasis on the immunologic aspects of the pathogenesis of endometriosis-associated infertility. To this end we called for a comprehensive and fundamental review of the involvement of immunology in reproduction.

As a whole these proceedings not only emphasize the complexity of the problem, but they raise some new concepts for the understanding of endometriosis-associated infertility.

We are grateful to Professor Masahiko Mizuno, Chairman of the Department of Obstetrics and Gynecology, University of Tokyo, for his advice and constant encouragement. We also thank all the contributors for their participation in the Second Hakone Conference on Endometriosis, and we gratefully acknowledge the educational grants from Tokyo Tanabe Co., Ltd., which made the conference and the publication of these proceedings possible.

The fetus as an allograft: Immunobiologic role of human trophoblasts for fetal survival

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OBJECTIVE: The hypothesis of this study is that trophoblasts contribute to the survival of the mammalian fetal allograft.

STUDY DESIGN: Immunologic and molecular biologic studies were performed with human trophoblasts or trophoblastic tumor cells to investigate the expression of class I human leukocyte antigens, the susceptibility to natural killer and lymphokine-activated cells, the ontogeny of Fc γ-receptors, and the production of immunosuppressive factors.

RESULTS: Heterogeneous expression of class I human leukocyte antigens on trophoblasts was regulated at transcriptional level. Trophoblasts showed low susceptibility to natural killer and lymphokine-activated cells. Trophoblasts expressed natural killer cell–type Fc γ-receptor III, which mediates phagocytosis. The trophoblast-derived immunosuppressive factor was very similar to transforming growth factor–β.

CONCLUSION: The trophoblasts, which ultimately form the fetoplacental interface, constitute a major immune barrier for the survival of the allogeneic conceptus. (Am J Obstet Gynecol 1992;167:251-6.)

Key words: Trophoblast, major histocompatibility complex, lymphokine-activated killer cell, Fc γ-receptor, immunosuppressive factor

Histocompatibility differences between a mother and her offspring in mammalian species create a paradox because the semiallogeneic fetus is not rejected by a competent maternal immune system during pregnancy. Several mechanisms that might be involved in the survival of the allogeneic conceptus have been suggested[1-7] (Table I). However, some of these hypotheses remain controversial and may represent only secondary or backup systems that contribute to the survival of the fetoplacental allograft. Among these mechanisms, recent studies on the immunology of pregnancy in humans and rodents have focused on the possible role of the placental trophoblast as an important immune barrier at the fetomaternal interface. In this article we review some immunologic functions of the trophoblast itself and various trophoblast-derived factors on the basis of data obtained in our laboratory. These findings can provide information for the analysis of the pathogenesis of endometriosis from the immunologic viewpoint, because available evidence indicates that endometriosis begins with the extrauterine implantation of endometrial cells or endometrial fragments that are transported through fallopian tubes or vascular channels.

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Heterogeneous expression of the human leukocyte antigen class I gene in human trophoblasts

The chorionic villi are enveloped by two layers of trophoblasts: the inner cytotrophoblasts and the outer syncytiotrophoblasts. Some extravillous cytotrophoblast cells migrate into the decidua and the myometrium. Thus a heterogeneous population of trophoblasts comes into close contact with various types of maternal tissue, including maternal blood and uterine elements, during pregnancy. Therefore the failure of trophoblast cells to demonstrate human leukocyte antigen (HLA) molecules may be one of the mechanisms that protects the semiallogeneic embryo from immunologic rejection by the mother.

Although HLA class I antigens are usually not detected on syncytiotrophoblasts or villous cytotrophoblasts, some extravillous cytotrophoblasts do express them.[3] However, the expression of these antigens by extravillous trophoblasts may be quantitatively different from the pattern of expression by other tissues. We used cultured trophoblastic tumor cells (SCH, JEG, BeWo, GCH1, NUC1, and HCCM5) as a model to investigate why trophoblasts do not demonstrate HLA antigens.

Various choriocarcinoma cell lines and two controls (RPMI 1788 and K562) were analyzed by cellular binding radioimmunoassay with use of monoclonal antibodies specific to HLA heavy chains and β_2-microglobulin. As shown in Table II, choriocarcinoma cells vary

Table I. Escape mechanisms of fetus/trophoblast from maternal immunity

1. Suppression of immune response by maternal lymphocytes
2. Poor immunogenicity of the trophoblast
3. Production of antiidiotypic antibodies
4. Production of blocking antibodies
5. Adsorption of antibodies by Fc receptors in the placenta
6. Blockade of afferent lymphatics by the decidua
7. Local immunoregulation caused by the products produced by the trophoblast and decidua
8. Reduced susceptibility of the trophoblast to immune lysis
9. Physiologic barrier of placenta to the passage of maternal and fetal cells.

Table II. Expression of HLA class I and β_2 microglobulin in choricarcinoma cells at translational and transcriptional levels

	HLA class I heavy chain		*β_2 microglobulin*	
Cell line	*Surface*	*Messenger RNA*	*Surface*	*Messenger RNA*
SCH	+ +	+ +	+ + +	+ + +
JEG	+	+	+ + +	+ + +
BeWo	−	+	+ + +	+ + +
GCH1	−	−	+ + +	+ + +
NUC1	+ / −	+ / −	+ + +	+ + +
HCCM5	+ / −	+	+ + +	+ + +
RPMI 1788	+ + +	+ + +	+ + +	+ + +
K562	−	−	+ + +	+ + +

widely in HLA class I surface antigens density.[8] SCH expresses approximately half the amount of HLA class I antigens compared with the B-lymphoblastoid cell line RPMI 1788. The expression of HLA class I antigens was weak on JEG, NUC1, and HCCM5, and no detectable levels of the antigens were observed on BeWo or GCH1 cells or on K562 cells that were used as the negative control. On the other hand, all the choriocarcinoma cells expressed a large amount of β_2-microglobulin on the cell surface. We analyzed the heterogeneity of HLA class I antigens expression in choriocarcinoma cell lines at the transcriptional level by Northern blot hybridization with use of phosphorus 32–labeled complementary deoxyribonucleic acid (DNA) probes specific for the HLA class I heavy chain and β_2-microglobulin. The levels of messenger ribonucleic acid (RNA) for the HLA class I heavy chain correlated well with the amounts of surface HLA class I antigens in all cell lines except BeWo. We detected a definite hybridizing band on the BeWo lane in spite of the absence of surface HLA class I antigens expression. Moreover, a band of truncated messenger RNA was observed in the BeWo lane which suggests an abnormality of class I heavy chain messenger RNA in BeWo. A recent study revealed that HLA-G antigen was expressed in first-trimester villous trophoblasts and that it was greatly reduced in third-trimester cytotrophoblasts. Among choriocarcinoma cell lines HLA-G antigen was expressed in JEG3 and BeWo but not in Jar.[9, 10] These findings indicate that the unique HLA class I antigens might be expressed in trophoblasts and choriocarcinomas.

The transcription of eukaryotic genes is usually regulated by the combination of *cis*-acting control elements and *trans*-acting cellular factors. Binding of nuclear proteins to regulatory elements can promote, enhance, or repress gene transcription. To study the differences in the control of major histocompatibility complex class I antigens expression among choriocarcinoma cell lines, we identified the human class I regulator elements located on the promoter region by their binding to nuclear proteins of the trophoblastic tumor cell lines.[11] By comparison of the murine and human major histocompatibility complex class I promoter region, we detected a human class I regulator element of 30 base pairs that was located 185 base pairs upstream of the HLA class I gene. This region contains core sequences that are identical to the murine class I regulator element described by Ozato et al.[12] A gel mobility shift assay was then performed to investigate the effect of nuclear factors binding to this regulator element. With use of nuclear extracts from SCH, JEG, HCCM5, and NUC1 cells that have HLA class I messenger RNA, we demonstrated that the human class I regulator element forms complexes on the polyacrylamide gel during electrophoresis. In the case of GCH1 or K562 cells no prominent DNA-protein complex was detected with use of this element. These results suggest that class I regulator element–nuclear protein interactions may be required for the transcription of HLA class I antigens on villous trophoblasts could be caused by the absence of this nuclear protein.

Impaired susceptibility of trophoblasts against major histocompatibility complex nonrestricted killer cells

The unique expression of major histocompatibility antigens on human trophoblast cells should allow the fetoplacental unit to escape recognition by the maternal immune system. Furthermore, the susceptibility of trophoblasts to class I–specific cytotoxic T lymphocytes is unique. Semiallogeneic and fully allogeneic trophoblast cells are resistant to allospecific T cell–mediated lysis.[13] The possibility exists that the placenta protects the fetus from maternal immune rejection through the resistance of trophoblast cells to major histocompatibility complex nonrestricted killer cells such as natural killer cells and lymphokine-activated killer cells. We have de-

Table III. Sensitivity of various human tumor cell lines to lymphokine-activated killer cells

Cell line	*Origin*	*Sensitivity to lymphokine-activated killer cells**
GCH1	Choriocarcinoma	+
GCH2	Choriocarcinoma	+
BeWo	Choriocarcinoma	+
HCCM5	Choriocarcinoma	+ +
SCH	Choriocarcinoma	+
K562	Non-B non-T cell	+ + + + +
Daudi	B cell	+ + + + +
RPMI 1788	B cell	+ + + +
SKW-3	T cell	+ + +
Scattola	Neuroblastoma cell	+ + +
Calu	Lung cancer	+ + +
Luci	Lung cancer	+ + + +
Panc 1	Pancreatic cancer	+ + + +
Lovo	Colon cancer	+ + + +
HeLa	Cervical cancer	+ + + + +
Ishikawa	Endometrial cancer	+ + +
Ono	Endometrial cancer	+ + + + +
Caki-1	Renal cancer	+ + + +
OS-RC-2	Renal cancer	+ + + + +
ACHN	Renal cancer	+ + + +
A498	Renal cancer	+ + +

(+) 0 to 20%; (+ +) 21% to 40%; (+ + +) 41% to 60%; (+ + + +) 61% to 80%; (+ + + + +) 81% to 100% specific lysis.
*A 4-hour chromium-releasing assay was performed at an E/T ratio of 20.

scribed an in vitro experiment indicating that the susceptibility of cultured trophoblastic tumor cell lines to natural killer and lymphokine-activated killer cells is impaired.[14]

A standard 4-hour chromium 51–releasing assay was used to measure the killing activity of natural killer or lymphokine-activated killer cells against target cells. Lymphokine-activated killer cells were generated by in vitro incubation of peripheral blood lymphocytes in a complete medium that contained recombinant interleukin-2. We then determined the antitumor effect of lymphokine-activated killer cells against several gynecologic tumor cell lines. The cell lines used were HeLa (cervical adenocarcinoma), Ishikawa and Ono (endometrial adenocarcinoma), and GCH1, GCH2, BeWo, and HCCM5 (choriocarcinoma). The three cell lines derived from uterine cancer, HeLa, Ishikawa, and Ono, were killed by either natural killer cells or lymphokine-activated killer cells. On the other hand, lymphokine-activated killer activity against gestational choriocarcinoma cell lines was definitely low. Moreover, natural killer activity against the choriocarcinoma cell lines was not detected at all. These results suggest that the sensitivity of choriocarcinoma to natural killer and lymphokine-activated killer cells is much lower than that of other gynecologic tumors. This was confirmed by a more extended experiment that used a wide panel of culture tumor cell lines as a target of lymphokine-activated killer activity (Table III). Among the 21 cell lines tested, the five choriocarcinoma cell lines, GCH1, GCH2, BeWo, HCCM5, and SCH, showed low levels of cytotoxic effect, and specific lysis did not exceed 20% except for HCCM5. On the other hand, the remaining 16 tumor cell lines, which included uterine, renal, colon, lung, and leukemia cells, showed substantial levels of lysis that exceeded 40%. It was concluded that choriocarcinoma cell lines are less sensitive to lymphokine-activated killer cells than the other cell lines tested in this experiment.

The impaired sensitivity of trophoblastic tumor cell lines to lymphokine-activated killer cells was confirmed by a cold inhibition experiment against lymphokine-activated killer activity. Various numbers of cells were added to a given number of chromium 51–labeled lymphokine-activated killer–sensitive Daudi cells and the lymphokine-activated killer activity was determined. Daudi and K562 cells competitively inhibited lymphokine-activated killer activity. However, the GCH1 choriocarcinoma cell line was less inhibitory. No significant inhibition was observed with the same number of inhibition cells as target cells, and only slight inhibition was seen with a fourfold excess of inhibitory cells.

We examined the effect of the trinitrophenol modification of target cells on the extent of lymphokine-activated killer cell–mediated lysis. A choriocarcinoma cell line, GCH1, was modified with trinitrophenyl and used as the target of recombinant interleukin-2–activated effector cells. As control experiments, trinitrophenol modified or unmodified autologous phytohemagglutinin blasts were simultaneously examined for their sensitivity to lymphokine-activated killer cells. As expected, autologous phytohemagglutinin blasts could be lysed substantially by lymphokine-activated killer cells, and this specific lysis was significantly enhanced

Table IV. Messenger RNA expression and localization of three classes of Fc γ receptors in human placenta

Fc γ receptor	*Messenger RNA expression*				*Localization*
	5W	*9W*	*23W*	*38W*	
Fc γ RI	−	+	+ +	+ +	Hofbauer cell
Fc γ RIIa	−	+	+ +	+ +	Endothelial cell
Fc γ RIIb	−	−	+ +	+ +	
Fc γ RIII	−	+	+ +	+ +	Trophoblast cell

by trinitrophenol modification of the target phytohemagglutinin blasts. On the other hand, trinitrophenol modification did not affect specific lysis of the choriocarcinoma cell line. Little lysis by lymphokine-activated killer cells was observed for either trinitrophenol-modified GCH1 cells or unmodified GCH1 cells. These results indicate that stearic hindrance of the lymphokine-activated killer target molecules expressed on trophoblast cells is unlikely. The impaired susceptibility of trophoblast cells to major histocompatibility complex nonrestricted killing, as demonstrated by these studies, should contribute to the survival of the semiallogeneic fetus.

Ontogeny and localization of Fc γ-receptors on the fetomaternal interface

Fc γ-receptors on the fetomaternal interface may act as multifunctional molecules, which may serve not only to transmit immunoglobulin G (IgG) but also to adsorb cytotoxic antibodies or to clear immune complexes.[5] Increasing evidence has recently indicated a previously unrecognized molecular and functional heterogeneity of the Fc γ-receptor. Three distinct classes of this receptor (Fc γ RI, Fc γ RII, and Fc γ RIII) have been defined on human myeloid cells by their reactivity with specific monoclonal antibodies.[15, 16] Fc γ RI (CD64) is a 70-kd glycoprotein found on monocytes and macrophages that binds human monometric IgG with a high affinity. Fc γ RII (CDw32) is a 40-kd glycoprotein receptor and is expressed on neutrophils, eosinophils, platelets, B cells, and monocytes. Fc γ-receptor RIII (CD16) has a different molecular structure and its own distinctive pattern of cellular distribution. It appears on macrophages, eosinophils, neutrophils, natural killer cells, killer cells, and some T cells. Fc γ RII and RIII are low affinity receptors for IgG and the demonstration of these Fc γ-receptors requires the presentation of ligands as a complex, either as a soluble IgG complex or as IgG-coated particles. Complementary DNA clones of these three classes of Fc γ-receptors have been isolated by several investigators since 1988 and are now available for the study of these receptors at the transcriptional level.

We used human placenta from different gestational weeks to demonstrate the expression of Fc γ-receptors at the fetomaternal interface. Four previously described complementary DNA clones, p135 (Fc γ RI), FcR2003 (Fc γ RIIa), FcR201 (Fc γ RIIb), and pCD16 (Fc γ RIII), were probed to demonstrate differential messenger RNA expression of Fc γ-receptors in human placental tissue at 5, 9, 23, and 38 gestational weeks (Table IV). The p135 probe hybridized the 1.7 kb messenger RNA of Fc γ RI in placenta from gestational weeks 9, 23, and 38; the pCD16 probe hybridized the 2.0 kb messenger RNA of Fc γ RIII in placenta from the same weeks as those used in the hybridization with p135. The transcriptional level of both receptors in the placenta was consistent through the first, second, and third trimesters of pregnancy. In contrast to Fc γ RI and RIII, two different transcripts, 2.4 kb and 1.6 kb of Fc γ RIIa, were detected in placenta from gestational weeks 9, 23, and 38. On the other hand, a 1.6 kb Fc γ RIIb transcript was recognized at gestational weeks 23 and 38 but not at gestational weeks 5 and 9 with the FcR201 probe, which indicates that the placenta expresses abundant Fc γ RII, especially Fc γ RIIb, in the placenta from the second and third trimester.[17]

We further investigated immunohistochemically the localization of the three Fc γ-receptors in placental tissue with specific monoclonal antibodies raised against them (Table IV). Monoclonal antibody 32.2 against Fc γ RI stained placental macrophages that have large nuclei and are known as Hofbauer cells, whereas positive staining of monoclonal antibody 2E1 for Fc γ RII was observed on the endothelial cells of small vessels on the fetal side. When we used monoclonal antibody Leu11b for Fc γ RIII, the outer layer containing syncytiotrophoblasts and cytotrophoblasts was stained. These results indicate that the Fc γ-receptors examined were localized at different sites within the placenta.[18]

Sequence analysis of the complementary DNA clones encoding placental messenger RNA from the Fc γ RIII receptor revealed that this fragment is a membrane-spanning molecule that has a 21 amino acid insertion and is located at the c terminus of Fc γ RIII on polymorphonuclear cells, resembling that on the natural killer cells.[19] Natural killer cell–type Fc γ RIII on placental trophoblasts might mediate phagocytosis. We

propose that these Fc γ-receptors located at different sites of the fetomaternal interface may act as multifunctional molecules that participate in the transmission of IgG and the clearance of immune complexes.

Trophoblast-derived immunosuppressive factor

A number of laboratories have demonstrated that trophoblast cells isolated from the placenta inhibit lymphocyte functions in vitro. This has led to the hypothesis that local immunoinhibition by a factor secreted by the trophoblast and operating only at the fetomaternal interface prevents maternal antifetal responses.

We have found a lymphocytostatic soluble factor secreted from cultured human trophoblasts.[20] Trophoblast cells prepared by collagenase digestion followed by differential centrifugation were cultured for a short time, and the culture supernatant was examined for its effect on in vitro lymphocyte functions. The supernatant suppressed the reaction of peripheral blood lymphocytes to mitogens and alloantigens. In addition, the supernatant reduced natural killer cell activity against K562 target cells. Moreover, the generation of cytotoxic T lymphocytes was markedly depressed.

Because we could not obtain large amounts of the immunosuppressive factor from human placenta, which is very unstable, we could not further explore the mechanisms of its immunosuppressive effect. However, while investigating the culture supernatants of choriocarcinoma cell lines, we found that these trophoblastic tumor cells produced the same suppressive factor produced by normal trophoblast cells.[21] The site of action of this factor in the T-cell signal transduction pathway was investigated with use of phorbol ester and a calcium ionophore (A23187), reagents that strongly stimulate the interleukin-2–mediated T-cell response, and we could demonstrate that this factor completely suppresses the human T-cell response activated by phorbol ester and A23187.[21-23] It failed to inhibit CD25 expression and interleukin-2 production by T-cell blasts in the T-cell activation phase, but it completely blocked the recombinant interleukin-2–induced proliferation of T-cell blasts in the T-cell blast proliferation phase. This factor acted on the cytoplasmic signal transduction pathways without interfering with the interleukin-2 molecules themselves or their interaction with interleukin-2 receptor. These findings suggest that the choriocarcinoma-derived factor only suppresses the proliferative function of immunocompetent cells and does not inhibit either activation or differentiation events.

At present, the choriocarcinoma-derived immunosuppressive factor has been neither purified nor identified as a single molecule. However, the comparison of this factor with several immunosuppressive agents indicates that it might be associated with the transforming growth factor–β (Table V). Transforming growth factor-β is a product of neoplastic and hematopoietic cells that is a functional regulator of the immune response. Transforming growth factor–β blocks the ability of interleukin-1 to stimulate lymphocyte proliferation. Besides, transforming growth factor–β_1 and its homologue, transforming growth factor–β_2, suppress interleukin-1-dependent murine thymocyte proliferation. Transforming growth factor–β also inhibits human peripheral blood T-lymphocyte mitogenesis. Inhibition of cell division appears to occur after activation of the lymphocytes, because neither the gene expression nor the translation of the interleukin-2 receptor is suppressed.[24] Furthermore, transforming growth factor–β does not block the synthesis of interleukin-2. These findings are very similar to the data we obtained on the mode of action of the choriocarcinoma-derived factor. We also found a significant level of latent transforming growth factor–β activity in defined trophoblast culture medium, as assayed by the inhibition of DNA synthesis in mink lung epithelial cells.[25]

Table V. Association of the choriocarcinoma-derived factor with immunosuppressive agents

Immunosuppressive agent	*Association with choriocarcinoma-derived factor*
α-Fetoprotein	No
Carcinomembryonic antigen	No
Human chorionic gonadotropin	Unlikely
Progesterone	Unlikely
Interferon-β	Unlikely
Ganglioside	No
Transforming growth factor–β	Probable

Comment

Within the placenta the fetal boundary consists entirely of trophoblastic material that is either exposed directly to maternal blood or in intimate apposition to endometrial cells. A central issue regarding the survival of the fetal allograft during pregnancy is therefore whether the trophoblast expresses those antigens coded for by the paternally inherited genes of the fetus. The unique regulation of HLA class I antigen expression by the interaction of the regulatory element with the nuclear proteins could be the important key to solve this question. The second role of the trophoblast for fetal survival against maternal immune attack is the low susceptibility of the trophoblast to either major histocompatibility complex restricted or nonrestricted immune cytolysis. In addition to their resistance to cell-mediated cytolysis, the trophoblast can adsorb cytotoxic antibodies or immune complexes by various subtypes of Fc γ-receptors expressed at different sites in the

placenta. Furthermore, soluble immunosuppressive molecules derived from the trophoblast could modulate immune reactivity in the periplacental environment. Thus the immunoregulatory mechanisms mentioned above appear to operate in harmony to regulate the maternal immune system and support fetal survival and growth during pregnancy.

Similar to the fetoplacental allograft, endometriosis is considered to be associated with altered immune function. Changes in both cell-mediated and humoral immunity in women who have endometriosis have been observed by several investigators.[26, 27] The presence of antibodies against endometrial tissue antigens in serum and peritoneal fluid from women with endometriosis could be caused by enhanced local immune reactivity.[28] Furthermore, the levels of antiendometrial antibodies in endometriosis were lowered by treatment with danazol or gonadotropin-releasing hormone agonists.[29, 30] These observations may lead to a reconsideration of the diagnostic and therapeutic approaches in endometriosis.

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Suppression of natural killer cell activity by sera from patients with endometriosis

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OBJECTIVE: We determined the effect of sera from patients who have endometriosis on natural killer cell activity.

STUDY DESIGN: The natural killer cell activity of lymphocytes from healthy volunteers was examined after incubation with sera from patients who had endometriosis or from controls, with K562 cells used as targets.

RESULTS: Lymphocytes treated with sera from patients who had endometriosis expressed significantly lower levels of cytotoxicity compared with lymphocytes treated with control sera. This suppression of cytotoxicity was dose dependent, and the degree of suppression was proportional to the incubation time of the effector cells with the sera. Decreased cytotoxicity after serum treatment was also observed with sera from patients who had been treated with danazol.

CONCLUSIONS: These findings show that humoral factors that can inhibit natural killer cell activity in vitro are present in the peripheral blood of patients who have endometriosis; moreover, they suggest that the suppressed natural killer cell activity may allow the development of endometrial cells at ectopic sites. (AM J OBSTET GYNECOL 1992;167:257-61.)

Key words: Endometriosis, natural killer cell activity, suppression, serum factors

Endometriosis is a well-tolerated autotransplantable disease that primarily affects women who are in their reproductive years. Retrograde spillage of menstrual blood into the peritoneal cavity occurs in most women who have patent fallopian tubes and is considered the leading cause of endometriosis.[1, 2] However, it is not known why endometriosis occurs in only a small percentage of susceptible women. Therefore it is possible that mechanisms that prevent the ectopic development of endometrial cells operate in resistant women.

Natural killer cells belong to a subpopulation of lymphocytes that represent the first line of defense against the initiation and spread of tumors in vivo.[3] Defective natural killer cell activity has been shown to predispose subjects to an enhanced growth of transplanted tumors.[4, 5] Diminished natural killer cell activity has also been found in tumor-bearing animals[6] and in humans who have malignant disease, especially in those who have widely disseminated tumors.[7] Therefore it is of great interest to know whether endometriosis is associated with certain aspects of natural killer cell activity believed to be important in the restriction of tumor growth in vivo. In this study we investigated the effect of sera obtained from fertile control subjects and from patients who had endometriosis on natural killer cell activity in vitro by a cytotoxicity assay. Natural killer–sensitive K562 cells were used as the target.

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6/0/38228

Material and methods

Serum samples. Serum samples were obtained from 10 healthy men and 10 fertile women who did not have endometriosis (control groups) and from 34 patients who had endometriosis (experimental group). Of the experimental group 9 patients were untreated, 12 were undergoing therapy with danazol (Bonzol, Tokyo Tanabe Co., Ltd., Tokyo, Japan), and 13 had completed treatment (9 patients received danazol therapy and 4 patients received danazol therapy followed by surgery). Blood samples in the group that had been treated were taken at least 2 months after the completion of treatment. The mean age of patients who had endometriosis before, during, and after danazol therapy was 30 years, 33 years, and 33 years, respectively. The mean age of the male and female control subjects was 31 years and 28 years, respectively. Serum samples were heat inactivated at 56° C for 30 minutes and stored at −20° C until use. Endometriosis was diagnosed by laparoscopic or laparotomic examination. None of the women included in this study had a malignant disease or had received any other hormone therapy, except for danazol, for at least 2 months before the serum collections.

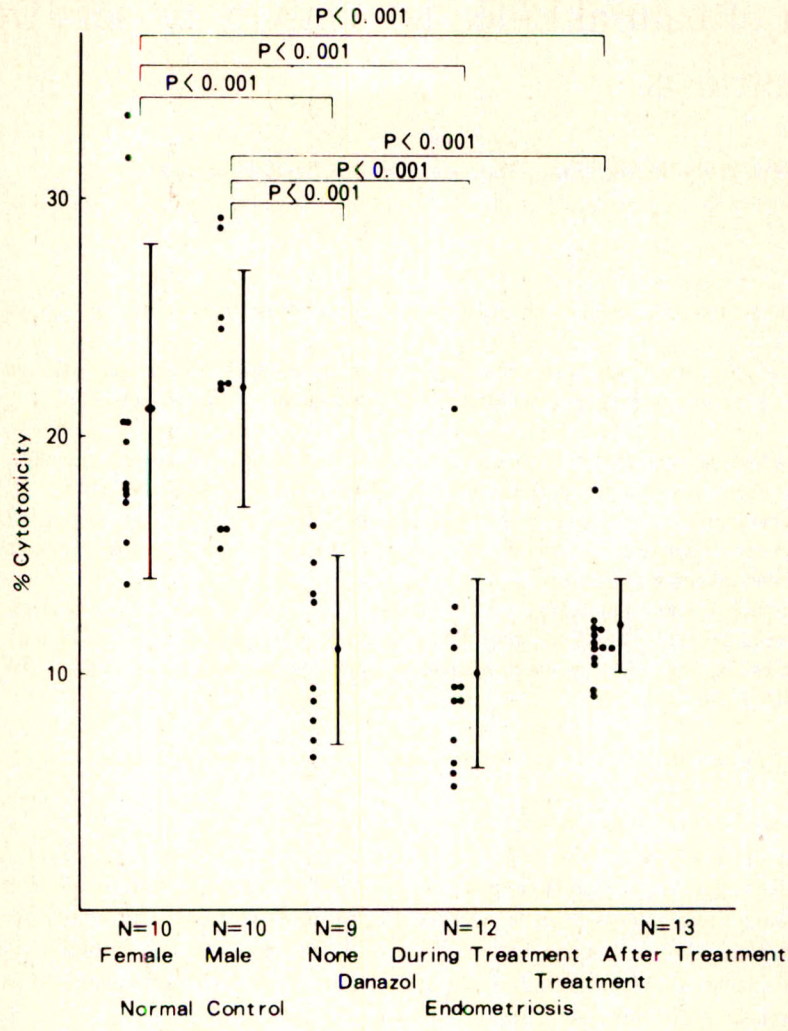


Fig. 1. Effects of peripheral blood sera on natural killer cell activity in vitro. Percent cytotoxicity after incubation with sera from 34 patients (9 before therapy, 12 during therapy with danazol, and 13 after therapy with danazol, surgery, or both) was significantly lower than after incubation with sera from male and female control subjects (n = 10 each).

Effector cells. Lymphocytes from two healthy men and two healthy women volunteers were separated by centrifugation on Ficoll-Hypaque gradients. The lymphocytes were suspended in RPMI-1640 medium (Gibco Laboratories, Grand Island, N.Y.) supplemented with 100 IU/ml of penicillin G and 100 μg/ml of streptomycin sulfate. The 600 μl aliquots of lymphocyte suspensions that contained 1.5 × 10^6 cells each were placed in round-bottomed tubes (12 × 75 mm, Falcon 2063, Becton Dickinson Labware, Lincoln Park, N.J.) and incubated with serum samples (40% vol/vol; total volume: 1 ml) previously sterilized through millipore (0.45 μm pore Millex HA filter unit; Millipore Co., Tokyo, Japan). After incubation at 37° C in a humidified atmosphere of 5% CO_2 and 95% in air for 18 hours the lymphocytes were washed three times and resuspended in RPMI-1640 medium supplemented with 100 IU/ml of penicillin G, 100 μg/ml of streptomycin sulfate, and 10% heat-inactivated fetal calf serum (Gibco) (complete medium). These serum-treated lymphocytes were usually more than 95% viable as assessed by the trypan blue dye exclusion test and were used as the effector cells.

Target cells. Human K562 myeloid leukemia cells were used as the target cells. They were labeled with 100 μCi $Na_2{}^{51}CrO_4$ (specific activity: 100 to 350 μCi/μg chromium; Radiochemical Center, Amersham, Buckinghamshire, England) and incubated at 37° C for 2 hours. The K562 cells were then washed three times, suspended in complete medium, and further incubated at 37° C for 1 hour. Finally, the cells were washed again and resuspended in complete medium to a concentration of 5 × 10^4 cells/ml.

Chromium 51–release cytotoxicity assay (natural

killer assay). The 100 μl of chromium 51–labeled cell suspension (5×10^3 cells) and 100 μl of effector cell suspension were mixed together in 96-well round-bottomed microtiter plates (Corning Glass Works, Corning, N.Y.) in triplicate to yield various effector to target cell ratios (40:1, 20:1, and 10:1). The plates were incubated at 37° C in a humidified atmosphere of 5% CO_2 and 95% in air for 4 hours. The supernatants were then harvested, and the radioactivity was measured in an autogamma scintillation counter (model ARC-301B, Aloka, Tokyo, Japan). The mean percent cytotoxicity for each assay and the mean percent suppression in comparison with controls were calculated by the following formulas:

Percent cytotoxicity =
[(test cpm − spontaneous cpm)/(maximum cpm − spontaneous cpm)] × 100

Percent suppression = [(% cytotoxicity in controls − % cytotoxicity in patient sera-treated cells)/% cytotoxicity in controls] × 100

The spontaneous release was measured in wells that contained only labeled K562 cells. The maximum release was measured in wells that contained 100 μl of labeled K562 cells suspension and 100 μl of 3% Triton-X100 (Sigma Diagnostics, St. Louis, Mo.). The spontaneous chromium 51 release was 3% to 12% of the total isotope count, and the maximum release varied from 80% to 90%.

Statistical analysis. The results were expressed as mean values ± SD, and the statistical significance was examined by the unpaired Student *t* test.

Results

The control lymphocytes from two men and two women who were treated for 18 hours with sera from patients who had endometriosis showed significantly decreased natural killer cell activity at all effector to target cell ratios. Fig. 1 shows a representative result of the cytotoxicity assay performed at a 40 to 1 effector to target ratio with lymphocytes from a healthy male donor. Peripheral sera from the 34 patients who had endometriosis showed significantly higher inhibition of natural killer cell activity than those from the women and men in the control groups. No difference was found in natural killer suppressive activity of sera among those obtained before ($n = 9$), during ($n = 12$), and after ($n = 13$) treatment with danazol (400 mg/day for 4 months). No correlation was found between the level of suppression and the severity of endometriosis (data not shown); moreover, the inhibition of natural killer cell activity depended on the concentration of serum in the reaction mixture (Fig. 2).

Experiments on the kinetics of the serum treatment revealed that the cytotoxicity of lymphocytes treated with serum from patients who had endometriosis was

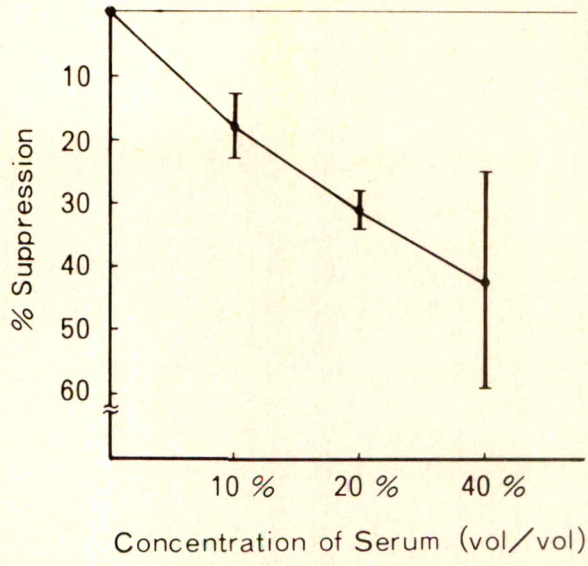


Fig. 2. Percent suppression of natural killer cell activity after an 18-hour incubation period with serum at various concentrations from a patient who had endometriosis.

significantly lower than that of lymphocytes treated with normal serum at every time point tested, except at 2 hours (Fig. 3). The percent inhibition increased when the serum treatment was prolonged from 2 hours to 27 hours. However, more than 27 hours were necessary to significantly decrease natural killer cell activity when control sera were used.

Comment

Several studies have suggested that endometriosis is accompanied by impaired immunity. In rhesus monkeys with spontaneous endometriosis cellular immune responses were reportedly depressed;[8] in humans, T-lymphocyte–mediated cytotoxicity to autologous endometrial cells was significantly reduced,[9] and lymphocytes stimulated by autologous endometrial antigens were also at a lower level in women who had endometriosis.[9]

Natural killer cells are considered the first line mechanisms of innate defense by which the host can prevent foreign cells from entering the body, surviving, or implanting themselves within the body. Natural killer cells can also control the development of neoplastic tumor cells. At present the role of natural killer cells in the occurrence or development of endometriosis is not fully understood, but it is reasonable to speculate that decreased natural killer activity may be associated with the onset or progression of this disease.

Oosterlynck et al.[10] recently reported that peripheral lymphocytes from women who had endometriosis had decreased natural killer activity against K562 cells and decreased cytotoxicity against both autologous and heterologous endometrial cells.[10] Our study demonstrated

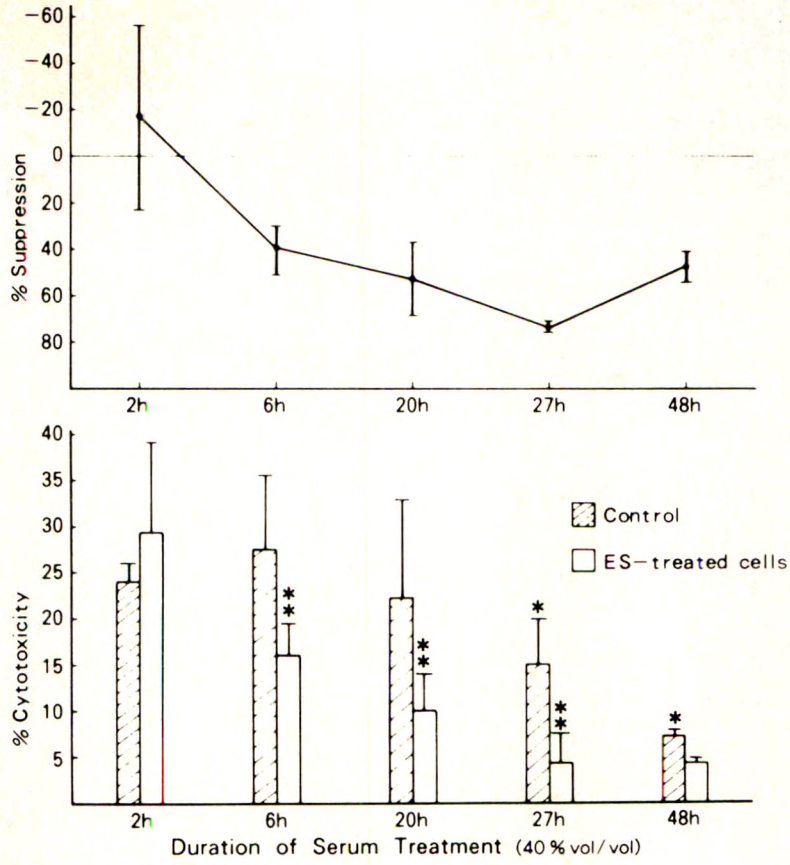


Fig. 3. Effect of the duration of serum treatment on natural killer cell activity. $*p < 0.01$ versus 2 hours. $**p < 0.01$ versus each control.

that serum obtained from patients who have endometriosis can inhibit natural killer cell activity in vitro. The spontaneous cytolytic activity of serum-treated lymphocytes against K562 cells was almost twofold greater with the use of sera from male or female control subjects than with the use of sera from patients who had endometriosis.

The decrease of natural killer cell activity induced by serum from patients who had endometriosis was not caused by the cytotoxicity of the sera, because the viability of the effector cells at the end of the serum treatment was usually more than 95%. Our previous studies demonstrated that the nondecidualized human endometrium releases some soluble factors that are capable of blocking lymphocyte proliferation in vitro[11, 12]; therefore it might be possible that the inhibition of natural killer cell activity was also mediated by soluble suppressive factors produced or released from the endometrial tissues.

In this study no difference was found in the percent suppression induced by sera obtained from patients before, during, and after treatment with danazol. In addition, no correlation was observed between the severity of the disease and the level of suppression. Therefore the serum factors that suppress natural killer cell activity probably do not derive directly from the ectopic endometrial cells themselves. To clarify the role of natural killer cells in the pathogenesis and prognosis of endometriosis it is necessary to know whether the serum natural killer cell suppression factors are present before the onset of the disease or are induced secondarily after the establishment of endometriosis.

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Significance of peritoneal macrophages on fertility in mice

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OBJECTIVE: To elucidate the role of peritoneal macrophages in infertility, we investigated how the presence of peritoneal macrophages would affect fecundity in mice. Moreover, we also studied the effects of interleukin-1 on embryonic development.

STUDY DESIGN: Mice were administered OK-432 intraperitoneally to induce macrophage infiltration of the peritoneal cavity; ovulation was then induced and animals were mated. On day 13 of gestation, fetuses were counted. After injection of OK-432 or interleukin-1, the mice were mated. Three days later, embryos were collected and the stage of embryo development was determined.

RESULTS: In mice given OK-432 ($n = 33$), four (12%) became pregnant and the mean litter number was 6.0 ± 3.6, whereas in the control group 23 of 30 mice (77%; $p < 0.01$) became pregnant and the litter number was 14.1 ± 5.3 ($p < 0.01$). OK-432 and interleukin-1 administered intraperitoneally significantly suppressed embryo development ($p < 0.01$).

CONCLUSION: Increased numbers of peritoneal macrophages negatively affect fecundity, probably by suppressing embryonic development. (Am J Obstet Gynecol 1992;167:261-4.)

Key words: Peritoneal macrophages, infertility, embryonic development, endometriosis

There are many infertile patients among even those with mild endometriosis,[1, 2] a disease of unknown origin. It is unclear how fecundity is affected in patients with mild endometriosis; some authors have postulated that the increased number[3, 4] and activity[5] of peritoneal macrophages in patients with endometriosis may be related to their decreased fecundity.

To elucidate the cause of infertility in patients with endometriosis, we investigated how the presence of peritoneal macrophages would affect embryonic development in mice. Moreover, we also studied the effects of interleukin-1, a humoral factor produced by peritoneal macrophages, on the embryonic development.

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Material and methods

Animals. ICR mice, 10 weeks old, were obtained from Clea Japan, Inc. (Tokyo, Japan).

Induction of macrophage infiltration into the peritoneal cavity. To cause macrophage infiltration into the peritoneal cavity we used OK-432 (Picibanil, Chugai Pharmaceutical Co., Tokyo, Japan) as the chemotactic factor. This is a lyophilized preparation of the penicillin-treated Su-strain of the *Streptococcus pyogenes* group A111.[6] OK-432 at a dose of 0.1 klinische Einheit was injected intraperitoneally. One klinische Einheit corresponded to 0.1 mg of the dried *Streptococci*. In a preliminary study the abdominal cavity was opened 3 days

Table I. Effect of OK432 intraperitoneal administration on animal fecundity

Group	*No.*	*Pregnancy rate*	*Litter No. (mean ± SD)*
OK-432	33	12% (4) *	6.00 ± 3.56 †
Control	30	77% (23) *	14.1 ± 5.26 †

*$p < 0.01$ (χ^2 test).
†$p < 0.01$ (Student t test).

Table II. Effect of prednisolone after OK-432 intraperitoneal administration on animal fecundity

Group	*No.*	*Pregnancy rate*	*Litter No. (mean ± SD)*
OK-432	33	12% (4) *	6.0 ± 3.6 †
OK-432 and prednisolone	21	43% (9) *	16.1 ± 8.7 †

*$p < 0.01$ (χ^2 test).
†$p < 0.05$ (Student t test).

later; 10 ml of physiologic saline solution was pipetted into the abdominal cavity and then aspirated. After centrifugation of the aspirate, the number of macrophages was counted. Macrophages were identified by a staining for α-naphthyl butyrate esterase.[7, 8]

Effect of OK-432 on ovulation rate. The effect of OK-432 on the ovulation rate was studied in another preliminary study in which the number of corpora lutea was counted. OK-432 was injected on day 1. Pregnant mare serum gonadotropin, 5 IU (Teikoku Zouki Co., Tokyo, Japan), was injected intraperitoneally on day 5, and human chorionic gonadotropin (hCG) (Mochida Pharmaceutical Co., Ltd., Tokyo, Japan) was injected on day 7 to induce ovulation. On the next day, both ovaries were removed, and the number of corpora lutea was counted.

Evaluation of fecundity. Fecundity of the mice was evaluated on the basis of the pregnancy rate and the number of fetuses. Ovulation was induced as previously described. Seven hours after the hCG injection, the animals were mated. The presence of a vaginal plug was then checked on the following morning, and if found, this was considered day 0 of gestation. On day 13 of gestation, the abdominal cavity was opened, and the fetuses, if any, were counted.

Study of the effects of OK-432 on fecundity. OK-432, pregnant mare serum gonadotropin, and hCG were injected as previously described. The day of the OK-432 injection was designed as day 1. Seven hours after the hCG injection, the animals were mated. On day 13 of gestation, the abdominal cavity was opened, and the fetuses, if any, were counted.

Study of the effects of prednisolone on fecundity after OK-432 administration. OK-432, pregnant mare serum gonadotropin, and hCG were injected, and the animals were treated as previously described. Prednisolone, 1 mg, was administered intraperitoneally on days 3, 4, and 6.

Study of the effects of OK-432 and interleukin-1 on embryonic development. After injection of OK-432, pregnant mare serum gonadotropin, and hCG, the animals were mated. In case of interleukin-1, recombinant human interleukin-1β (Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan), at a dose of 100 pg (1 μg/12.1 Japan reference unit), was administered intraperitoneally 5 hours after administration of hCG. Recombinant interleukin-1 was prepared in *Escherichia coli* and purified according to the method of Hirai et al.[9] The endotoxin level after purification was less than 100 pg/mg of protein. Ninety-six hours after hCG injection, the abdominal cavity was opened, and both uterine horns were extirpated. The uterine cavum was flushed with 0.2 ml of physiologic saline solution. Cleavage of embryo in flushed saline solution was examined under a contrast phase microscope. The results were expressed as the total number of blastocysts and morula divided by the total number of embryos.

Statistical analysis. The significance of the differences between two groups was evaluated by the Student t test or χ^2 test. When the results from the three groups were compared they were analyzed by Dunnett's multiple comparison test.

Results

After OK-432 administration, 95% of the leukocytes infiltrating the peritoneal cavity were macrophages. The mean number of macrophages after OK-432 administration to 20 mice [$(55.2 \pm 23.5) \times 10^5$] was significantly higher ($p < 0.01$) than in the 10 control mice injected with saline solution only [$(8.1 \pm 6.3) \times 10^5$]. The number of corpora lutea was not significantly different in the OK-432 group ($n = 3$; 24.3 ± 5.0 per mouse) and control group ($n = 3$; 24.5 ± 5.0 per mouse).

In the 33 mice given OK-432, the pregnancy rate was 12%, and the mean litter number was 6.0 ± 3.6 (± SD), that is, lower than in the (77% and 14.1 ± 5.3, respectively) 30 control mice; the difference was significant ($p < 0.01$) for the pregnancy rate and the litter number (Table I).

The pregnancy rate increased ($p < 0.01$) and the litter number was larger ($p < 0.05$) in mice given OK-432 and prednisolone than in those mice given OK-432 alone (Table II).

Regarding the effects of the intraperitoneal administration of OK-432 and interleukin-1 on embryonic development, the number of embryos that had reached the blastocyst or morula stages in the OK-432 group

Table III. Effect of OK-432 and interleukin-1 on embryo development

Group	No.	$\frac{\text{Blastocyst and morula}}{\text{All zygotes}} \times 100\ (\%)$		
OK-432	15	28.9 ± 22.9†		
Interleukin-1	15	39.3 ± 12.7	$p < 0.01$*	$p < 0.01$*
Control	15	65.3 ± 18.6		

*Dunnet's multiple comparison test.
†Data are expressed as mean ± SD.

($n = 15$) was 28.9 ± 22.9 and 39.3 ± 12.7 in the interleukin-1 group ($n = 15$); both were markedly lower ($p < 0.01$) than the number of blastocysts and morula in the control group (65.3 ± 18.5; $n = 15$) (Table III).

Comment

This study was undertaken to evaluate the effects of peritoneal macrophages on fertility with use of a mice model. OK-432 is used as a biologic response modifier and is also known as an activator of macrophages and natural killer cells[10]; we used it here to cause macrophage infiltration of the peritoneal cavity.

For the identification of macrophages, α-naphthyl butyrate esterase staining was used. With the technologic advances, a new technique that uses monoclonal antibodies has been developed for the identification of macrophages. However, it has been reported that with nonspecific esterase staining, such as the α-naphthyl butyrate esterase staining used in this study, the results obtained are the same as those obtained with monoclonal antibody.[11] In this study 95% of peritoneal leukocytes were macrophages; however, small numbers of lymphocytes and natural killer cells were included. We first studied the effects of OK-432 on ovulation, because prostaglandins secreted from macrophages are known to affect ovulation.[12] According to our observation, however, the number of corpora lutea was not different in mice given OK-432 or physiologic saline only after ovulation induced by pregnant mare serum gonadotropin and hCG, which suggests that these treatments do not interfere with ovulation.

The mice given OK-432 had decreased fecundity, which may suggest that the macrophages infiltrating the abdominal cavity interfere with fecundity. Prednisolone was then given to determine whether a decrease in the macrophage activity with the abdominal cavity would help restore fecundity; both the pregnancy rate and the number of fetuses increased. These results strongly suggested that the large number of macrophages observed in the peritoneal fluid of patients with endometriosis may contribute to infertility in these patients.

To evaluate the participation of macrophages on the decrease of fecundity, we studied their effects on embryonic development. Only a small number of fertilized eggs reached the stage of blastocyst or morula in the OK-432 group, which indicates that macrophages have an adverse effect on the growth of embryos.

On the basis of these results, we can say that the presence of macrophages in the abdominal cavity negatively affects fecundity and that their adverse effect on embryonic development is possibly one of the main etiologic factors involved in the decrease in fecundity.

In mice the ovary and uterus are connected; therefore neither the ovum, the sperm, or the embryo could have direct contact with the macrophages as it occurs in humans. It is then presumed that these adverse effects could have been caused by a humoral factor derived from macrophages.

Hence we examined the possible effects of interleukin-1, which is present at high concentrations in the peritoneal fluid of patients with endometriosis.[13, 14] The dose of recombinant interleukin-1β used here was 100 pg, because at this dose level we observed inhibition of fecundity in our previous study.[14] As a result, embryonic development was suppressed by recombinant interleukin-1β administration.

The adverse effects of peritoneal fluid of patients with endometriosis on embryonic development have been reported by Morcos et al.,[15] although their research was based on data obtained from in vitro fertilization. Similar reports were made by Fakih et al.[13] and Hill et al.[16] However, a recent study by Schneider et al.[17] showed that purified interleukin-1 had no effect on embryonic development.

Although our experiments were carried out in vivo, in view of the results reported by Schneider et al.,[17] we have to consider not the activity of interleukin-1 per se but also the possible participation of other cytokines such as prostaglandins and interleukin-6, which would increase after the administration of interleukin-1.

On the basis of the data presented here, it can be said that peritoneal macrophages have an adverse effect on fecundity and that they probably suppress embryonic development.

The restoration of fecundity after treatment with prednisolone suggested that prednisolone or any other immunosuppressive agent might be beneficial in the treatment of infertility associated with endometriosis, especially in those patients who do not respond to da-

nazol or gonadotropin-releasing hormone agonists alone, because prednisolone does not interfere with ovulation. Furthermore, it is presumed that this experimental model may be useful in the research of the mechanisms involved in infertility associated with endometriosis.

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Comparison of cytokine levels and embryo toxicity in peritoneal fluid in infertile women with untreated or treated endometriosis

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OBJECTIVE: Our aim was to examine the relationship between the levels of cytokines in peritoneal fluid and its embryo toxicity.
STUDY DESIGN: The levels of interleukin-1 and tumor necrosis factor were measured in peritoneal fluid from infertile women who did not have endometriosis (n = 21), who had untreated endometriosis (n = 19), and who had undergone medical treatment for endometriosis (n = 10). Embryo toxicity was investigated in mouse two-cell embryos cocultured with the oviducts in culture media that contained various concentrations of peritoneal fluid.
RESULTS: The levels of cytokines were significantly higher in the peritoneal fluid from women who had untreated endometriosis than in women who did not have endometriosis, but they were extremely low in women who had undergone medical treatment with either danazol or buserelin. The peritoneal fluid from women who had untreated endometriosis adversely affected the cleavage of mouse two-cell embryos. After medical treatment the embryo toxicity of the peritoneal fluid was almost undetectable.
CONCLUSION: These results offer some theoretic bases in support of medical treatment to improve reproductive performance in infertile women who have endometriosis. (Am J Obstet Gynecol 1992;167: 265-70.)

Key words: Endometriosis, peritoneal fluid, cytokines, embryo toxicity, medical treatment

Endometriosis is strongly associated with infertility. Reproductive performance is impaired even in women who have minimal and mild endometriosis and who are found to have no apparent anatomic derangement that might hinder early reproductive events, such as ovum pick up or its transport.[1, 2] Thus far diverse plausible mechanisms have been postulated to explain the lessened fecundity associated with endometriosis, including luteinized unruptured follicle,[3] anovulation,[4] luteal phase defect,[5] and impaired glycogen synthesis in the endometrium,[6] among others.

Several reports have recently pointed to the chronic inflammatory changes in the peritoneal cavity of women who have endometriosis, which are manifested by an increased volume of peritoneal fluid[7] and an increased number of peritoneal macrophages and their activities.[8, 9] Furthermore, it has been reported that macrophages from patients who have endometriosis release significantly higher tumor necrosis factor activity than those from fertile women who do not have endometriosis[10]; in addition, elevated levels of interleukin-1[11] and tumor necrosis factor[10, 12, 13] have been demonstrated in the peritoneal fluid of patients who have endometriosis. Because peritoneal fluid is supposed to go into the oviductal cavity, it may influence the early reproductive processes by modulating the microenvironment that surrounds the embryos. In this regard we have demonstrated that the peritoneal fluid from women who have endometriosis inhibits the cleavage of mouse early embryos by acting on the oviducts; thus it is possible that factors in the peritoneal fluid from these women are involved in the pathogenesis of infertility associated with endometriosis.[14] More specifically, we have suggested that elevated interleukin-1[14] and tumor necrosis factor[13] in the peritoneal fluid may be responsible for the embryo toxicity of the peritoneal fluid from women who have endometriosis.

These observations prompted us to investigate whether medical treatment of endometriosis reduces the levels of cytokines in peritoneal fluid and its effect on embryonic development. In this study we offer evidence that medical treatment of endometriosis decreases the cytokine levels and concurrently eliminates the embryo toxicity of the peritoneal fluid from women who have endometriosis.

Material and methods

Patients. The subjects in this study were 50 infertile women who had normal ovulatory cycles. Peritoneal fluid was collected during laparoscopy or laparotomy either for an evaluation of infertility or for treatment of infertility by means of tubal microsurgery. Twenty-

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Table I. Clinical profile of the women studied

	Nonendometriosis	*Untreated endometriosis*	*Treated endometriosis*
No. of cases	21	19	10
Age (mean ± standard error of mean)	32.5 ± 5.1	30.8 ± 3.8	31.9 ± 4.0
Duration of infertility	5.2 ± 3.0	4.6 ± 2.5	4.9 ± 2.7

Table II. Volume of peritoneal fluid

	Peritoneal fluid (ml)
Nonendometriosis	10.6 ± 8.5*
Untreated endometriosis	19.6 ± 9.0
Treated endometriosis	6.0 ± 4.1**

Values are mean ± standard error of the mean.
*$p < 0.05$ versus untreated endometriosis.
**$p < 0.01$ versus untreated endometriosis.

nine women had endometriosis. Of these, 19 (revised American Fertility Society stage I/II = 10; stage III = 3; and stage IV = 6) had not undergone any treatment for endometriosis, and 10 (stage I/II = 2; stage III = 4; and stage IV = 4) had received medical treatment for 4 to 6 months (4 with danazol [Bonzol, Tokyo Tanabe Co., Ltd., Tokyo, Japan], 400 mg/day, and 6 with intranasal buserelin [Suprecur, Hoechst Japan Ltd., Tokyo, Japan], 900 μg/day). The remaining 21 women had no evidence of endometriosis.

Collection of peritoneal fluid. Peritoneal fluid samples were obtained from each woman in the late proliferative phase by aspirating the fluid in the anterior uterovesical space and cul-de-sac. Peritoneal fluid samples from women who had endometriosis that had been treated were obtained 3 months after the termination of treatment. The fluid was centrifuged at 600*g* for 15 minutes, and the resultant cell-free supernatant was stored at −80° C until use.

Measurement of cytokines. The concentration of interleukin-1β in peritoneal fluid was measured with enzyme-linked immunosorbent assay kits (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan). The lowest measurable concentration was 2 pg/ml. The intraassay coefficient of variation was 4%, whereas the interassay coefficient of variation was 8.2%. The concentration of tumor necrosis factor−α was measured with enzyme-linked immunosorbent assay kits (Otsuka). The lowest measurable concentration was 4 pg/ml. The intraassay and interassay coefficients of variation were 3.1% and 12.0%, respectively.

Effect of peritoneal fluid on embryonic development. In a previous report[14] we have shown that the peritoneal fluid from women who have endometriosis inhibits the cleavage of mouse two-cell embryos cocultured with the oviducts. Therefore it was suggested that the peritoneal fluid from women who have endometriosis may primarily act on the oviducts and stimulate the release of certain humoral factors that in turn adversely affect embryonic development. In this study we used the same in vitro system to assess the embryo toxicity of the peritoneal fluid. Briefly, 8-week-old ICR mice were injected with 5 IU of pregnant mare serum (Teikoku Hormone Manufacturing Co., Ltd., Tokyo, Japan) intraperitoneally to induce superovulation, followed by an intraperitoneal injection of 5 IU of human chorionic gonadotropin (Teikoku) 48 hours later. Mating was confirmed by the presence of the copulatory plug. The oviducts were taken 48 hours after the human chorionic gonadotropin injection to obtain two-cell embryos. The embryos were cultured for 24 hours at 37° C under the condition of 95% air and 5% CO_2 in modified Biggers-Whitten-Whittingham medium in the presence of a piece of oviductal tissue (approximately 5 mm in length). The oviductal tissue was taken from nonpregnant 8-week-old Crj:CD-1 mice. The presence of the oviductal tissue did not affect the pH or the osmotic pressure of the culture media. Usually 10 embryos were cultured in a plastic organ culture dish (Becton Dickinson Labware, Lincoln Park, N.J.) that contained 1.5 ml of the medium. The maximum concentration of peritoneal fluid from women who did not have endometriosis at which no adverse effect was observed on embryonic development in vitro was 5%. Therefore this concentration was used throughout the experiments. From 10 to 30 embryos were used to assess the effect of each sample. The degree of development of cultured embryos was determined by microscopic observation.

Statistics. Data regarding cytokines were expressed as the mean ± standard error of the mean and analyzed by the Student *t* test. The Mann-Whitney U test was used for the analysis of embryonic development. A possibility of 0.05 was considered significant.

Results

Table I summarizes the clinical profiles of the women who participated in this study. Their mean age and the duration of infertility were essentially the same among the three groups (nonendometriosis, untreated endometriosis, and endometriosis after medical treatment).

Table II compares the volume of aspirated peritoneal fluid. The volume of peritoneal fluid in the untreated

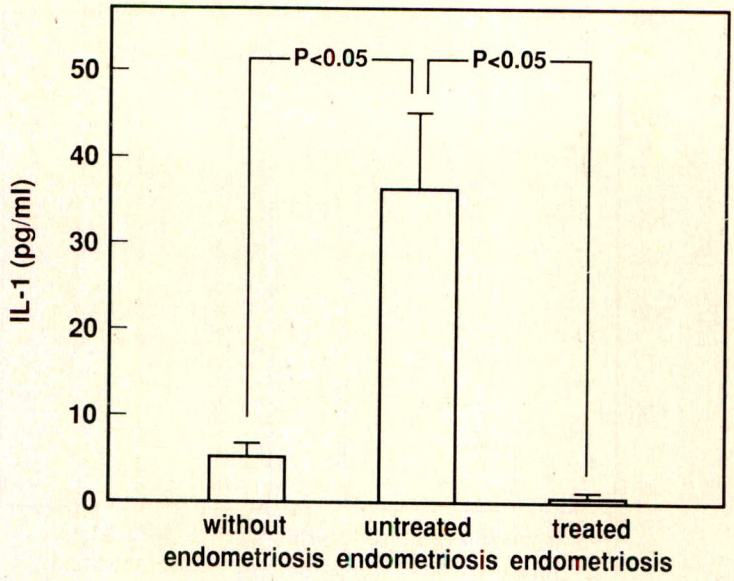


Fig. 1. Concentration of interleukin-1 in peritoneal fluid from women who did not have endometriosis, who had untreated endometriosis, and who had treated endometriosis. Each value represents the mean ± standard error of the mean.

endometriosis group was about twofold the volume found in the nonendometriosis group. However, the volume of peritoneal fluid was markedly lower in women who had undergone medical treatment; it was less than one third of the volume found in the untreated endometriosis group.

Interkeukin-1 levels in the peritoneal fluid from women who had untreated endometriosis were significantly higher than interleukin-1 levels in the nonendometriosis group. Notably, the interleukin-1 levels in women who had been treated for endometriosis were almost below detectability in all cases except one (Fig. 1). The same phenomenon was observed in the case of tumor necrosis factor (Fig. 2). The tumor necrosis factor levels in the peritoneal fluid from women who had untreated endometriosis were about fivefold the levels detected in the endometriosis group. The tumor necrosis factor levels were extremely low in the treated endometriosis group.

Fig. 3 illustrates the distribution of embryos according to their embryonal stage after they were cultured for 24 hours in medium that contained peritoneal fluid from women in each study group. Among the embryos cultured with peritoneal fluid from women who did not have endometriosis, 5- to 8-cell and 16-cell or more advanced stages accounted for 50% and 30% of the cultured embryos, respectively. In contrast, the percentage of 5- to 8-cell and 16-cell or more advanced stages were 33% and 13%, respectively, when 2-cell embryos were incubated with the peritoneal fluid from women who had untreated endometriosis. The analysis of the overall distribution between the two groups demonstrated that the embryonal development of embryos incubated with peritoneal fluid from women who had untreated endometriosis was significantly delayed ($p < 0.001$). It is noteworthy that the rate of degenerated embryos was significantly higher ($p < 0.001$) when embryos were incubated with the peritoneal fluid from women who had untreated endometriosis (25%) than that found when embryos were incubated with the peritoneal fluid from women who did not have endometriosis (8%). The peritoneal fluid from women who had treated endometriosis showed markedly reduced embryo toxicity compared with the peritoneal fluid from women who had untreated endometriosis; however, the rate of degenerated embryos was still higher (20%) compared with the rate found when embryos were incubated in the presence of the peritoneal fluid from women who did not have endometriosis ($p < 0.001$). Hence, regarding the overall distribution of the embryonal stages, a significant difference was found between the peritoneal fluid from women who did not have endometriosis and the peritoneal fluid from women who had treated endometriosis ($p < 0.01$).

We then examined the effect of each peritoneal fluid sample on embryonal development (Table III). Because the lowest percentage of embryos that developed into five-cell or more advanced stages was about 70% when two-cell embryos were incubated without peritoneal fluid, we regarded peritoneal fluid samples that produced over 70% embryos of five-cell or more advanced as nonembryo toxic. According to this definition 3 of 11 (18%) peritoneal fluid samples from women who

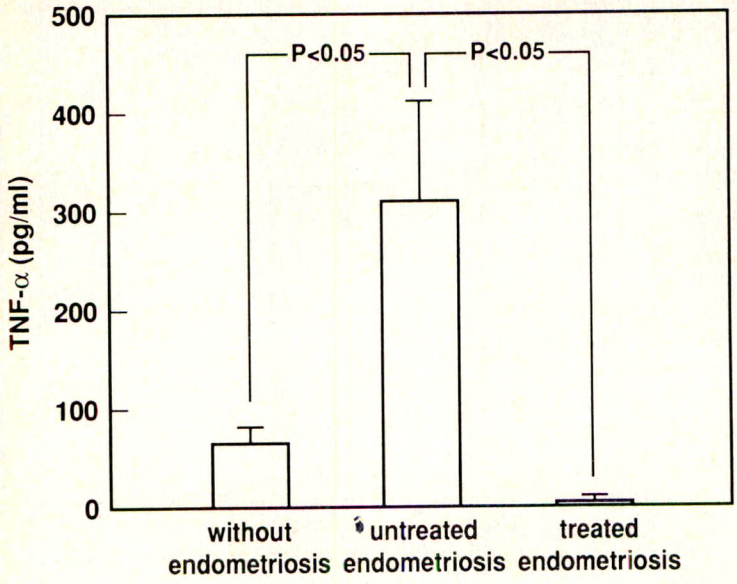


Fig. 2. Concentration of tumor necrosis factor in peritoneal fluid from women who did not have endometriosis, who had untreated endometriosis, and who had treated endometriosis. Each value represents the mean ± standard error of the mean.

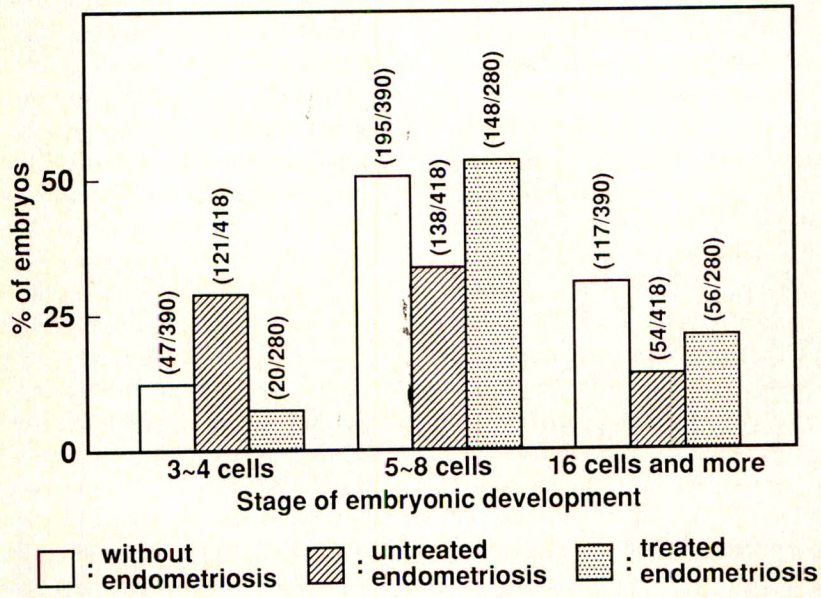


Fig. 3. Number of embryos at different stages of embryonic development after 24-hour incubation of mouse two-cell embryos with peritoneal fluid. Denominators correspond to the number of embryos examined and numerators correspond to the number of embryos at each stage of embryonic development. The remaining embryos were degenerated.

did not have endometriosis were embryo toxic, whereas 15 of 19 (79%) peritoneal fluid samples from women who had untreated endometriosis were embryo toxic; thus the incidence of embryo toxic peritoneal fluid was significantly different in these two groups ($p < 0.05$). However, only 20% of the peritoneal fluid samples in the treated endometriosis group were embryo toxic; that is, almost no difference was found in the occurrence of peritoneal fluid with embryo toxicity between the nonendometriosis group and the treated endometriosis group.

Comment

The main finding of this study is that the cytokine levels were markedly low in the peritoneal fluid from women who had undergone medical treatment for en-

dometriosis. The peritoneal fluid of these women also showed much less embryo toxicity compared with the peritoneal fluid from women who had untreated endometriosis. These results offer a theoretic background in favor of medical treatment for endometriosis-associated infertility.

A considerable body of evidence suggesting that intraperitoneal chronic inflammatory changes are presumptive etiologic factors for endometriosis-associated infertility has been accumulated. For instance, peritoneal fluid from patients who have endometriosis and increased levels of tumor necrosis factor reduces sperm motility.[15] In addition, peritoneal fluid from women who have endometriosis has been shown to exert a deleterious effect on sperm-oocyte interaction in mice.[16] Furthermore, Morcos et al.[17] reported that peritoneal fluid from women who have endometriosis inhibits the cleavage of mouse embryos. However, Awadalla et al.[18] failed to duplicate this finding. We were also unable to demonstrate a direct toxic effect of peritoneal fluid on embryonic development.[14] These conflicting results may be partly caused by the different species of mice or culture media that were used. Because peritoneal fluid is in direct contact with the oviductal cavity, we wondered whether peritoneal fluid could hinder embryonic development by altering the intraoviductal microenvironment that surrounds embryos at an early stage. Thus we cocultured the embryos with the oviducts in the presence of peritoneal fluid from infertile women who had endometriosis and were able to demonstrate the inhibition of embryonic development by the peritoneal fluid.[14] In this study we have confirmed our previous findings with use of the same experimental system. It is especially interesting to note that the embryo toxicity of peritoneal fluid was hardly detected after medical treatment. However, some of the peritoneal fluid samples still showed some degree of embryo toxicity after the treatment.

Cytokines, such as interleukin-1 or tumor necrosis factor, are usually found in high amounts in the peritoneal fluid from women who have endometriosis.[10-13] In our previous study[14] interleukin-1, like peritoneal fluid, inhibited embryonic development in the presence of the oviducts. Interestingly, indomethacin, which is an inhibitor of prostaglandin synthesis, effectively abolished the inhibitory effect of interleukin-1 and the peritoneal fluid on embryonic development. These findings suggested that interleukin-1, at increased concentrations, could contribute to the embryo toxicity of the peritoneal fluid from women who have endometriosis. The same was true for tumor necrosis factor.[13] Moreover, we showed that prostaglandin E_2 directly inhibits embryonic development in the absence of the oviducts.[14] Accordingly, prostaglandin E_2, which is released from the oviductal tissues through stimulation with peritoneal fluid,[14] appears to be one of the mediators in the pathogenesis of endometriosis-associated infertility. Notably, in this study we have demonstrated that the decrease in embryo toxicity of the peritoneal fluid from women who have treated endometriosis was accompanied by decreased cytokine levels. This finding provides strong support for the aforementioned suggestion that increased levels of cytokines in the peritoneal fluid may be involved in the pathogenesis of endometriosis-associated infertility.

Table III. Incidence of embryo toxic peritoneal fluid among women in the three groups studied

	No. of women with embryo toxic peritoneal fluid	*(%)*
Nonendometriosis	2/11*	18
Untreated endometriosis	15/19	79
Treated endometriosis	2/10*	20

Mouse two-cell embryos were incubated for 24 hours with 10% of each peritoneal fluid sample. Peritoneal fluid samples that could not produce 70% of embryos of five cells or more were regarded as embryo toxic.

*$p < 0.05$ versus untreated endometriosis.

The question remains as to whether the inflammatory changes in the peritoneal cavity are a consequence of the presence of endometriotic tissues. In this study cytokine levels were markedly lower in the peritoneal fluid from women who had treated endometriosis. This finding appears to support the viewpoint that the presence of endometriotic tissues evokes inflammatory changes in the peritoneum. The possibility remains that the drugs used in this study directly modulate the cytokine production in a manner unrelated to their clinical efficacy. Indeed, danazol has been shown to have immunosuppressive effects in vitro.[19] Moreover, danazol, but not buserelin, suppresses autoantibodies in women who have endometriosis.[20] Thus it cannot be ruled out that danazol may directly modulate the production of cytokines by macrophages. However, both danazol and buserelin were associated with low cytokine levels. In addition, it has been reported that treatment of endometriosis with medroxyprogesterone reduces intraperitoneal inflammatory changes, which is exemplified by a reduced peritoneal fluid volume and lower leukocyte counts in the peritoneal fluid of those patients.[21] Therefore it appears that the reduced cytokine levels observed in the peritoneal fluid from women who have treated endometriosis are presumably caused by a reduction of endometriotic tissues but not by the direct effect of the drug used.

The data obtained in this study provide some insight into the causal relationship between endometriosis and infertility. Regarding the embryo toxicity of the peritoneal fluid, results from this study suggest that the presence of endometriosis adversely affects fecundity.

However, as yet the beneficial effect of medical treatment in improving fecundity in women who have endometriosis has not been proved. A likely explanation for this is that divergent mechanisms may be involved in endometriosis-associated infertility and medical treatment cannot eliminate every possible etiologic factor of infertility. Alternatively, our results showed that degenerated embryos were still found more frequently among embryos incubated with peritoneal fluid from women who had treated endometriosis compared with those incubated with peritoneal fluid from women who did not have endometriosis. It thus appears feasible that unidentified factors involved in the degeneration of embryos may remain in the peritoneal fluid from women who have treated endometriosis. We are currently investigating the presence of such factors.

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Luteinized unruptured follicle in the early stages of endometriosis as a cause of unexplained infertility

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OBJECTIVE: We attempted to clarify the relationship between luteinized unruptured follicle, which occurs in the early stages of endometriosis, and unexplained infertility.

STUDY DESIGN: Seventy patients who had unexplained infertility were reviewed.

RESULTS: Laparoscopic examination showed that 47 patients (67%) had endometriosis; of these, 40 (85%) had minimal or mild disease. The incidence of luteinized unruptured follicle was higher ($p < 0.05$) in patients who had endometriosis (35%/patient and 25%/cycle) compared with patients who did not have endometriosis (11%/patient and 7%/cycle). Degenerated oocyte cumuli were collected in 6 (43%) of 14 luteinized unruptured follicles diagnosed by transvaginal ultrasound.

CONCLUSIONS: These results show that luteinized unruptured follicle is common in patients who have mild or minimal endometriosis and that it may be one of the causes of endometriosis-associated infertility. Transvaginal ultrasound-guided follicular puncture of luteinized unruptured follicle during the mid luteal phase may be useful in establishing a definitive diagnosis of luteinized unruptured follicle. (AM J OBSTET GYNECOL 1992;167:271-3.)

Key words: Luteinized unruptured follicle, endometriosis, unexplained infertility, ultrasonography

Endometriosis is an enigmatic disease that is frequently associated with infertility. In the advanced stages of the disease infertility is easy to explain because of the presence of adhesions, ovarian endometrioma, or both, but a direct cause-and-effect relationship in the early stages of endometriosis is not fully understood. Among the mechanisms that might be involved in endometriosis-associated infertility is the luteinized unruptured follicle.[1] Originally described by Jewelewicz,[2] the luteinized unruptured follicle may be one of the causes of infertility in women who have endometriosis,[3-6] although its significance is still controversial.[7-9] In our fertility clinic minimal or mild endometriosis was demonstrated on laparoscopy in more than 50% of patients who had unexplained infertility. Moreover, luteinized unruptured follicle was frequently found by ultrasonic examination in patients who had unexplained infertility.

This study was undertaken to elucidate whether luteinized unruptured follicle is associated with endometriosis in the early stages of the disease and to investigate its potential role in endometriosis-associated infertility. We discuss the results in relation to the relevant published reports available.

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Material and methods

Between May 1989 and April 1991, 146 patients who desired to have a child underwent diagnostic laparoscopic examination and a tubal dye test in our clinic. Seventy of these 146 patients had received a diagnosis of unexplained infertility before laparoscopic examination, because their test results from the routine laboratory workup for couples with infertility were normal. This routine laboratory workup included follicular monitoring by ultrasonography. From these 70 patients a study group of 47 patients who had endometriosis was selected. Of the remaining patients who had normal findings, 28 had husbands with a low semen analysis; these patients constituted the control group in this report.

Documentation of endometriosis. We performed all laparoscopies with use of the double-puncture technique, with the patients under general anesthesia, on day 7 to day 10 of the menstrual cycle. The stage of endometriosis was determined according to the weighted point system of the Revised American Fertility Society Classification of Endometriosis.[10] Unipolar electrocautery was performed when minimal or mild endometriosis was observed. When we believed that electrocautery would not be effective, no attempt was made to excise or cauterize the endometriotic lesion. In such cases medical therapy with danazol (Bonzol, Tokyo Tanabe Co., Ltd., Tokyo, Japan) or gonadotropin-releasing hormone analog (buserelin; Suprecur, Hoechst Japan, Tokyo, Japan) was chosen for further treatment.

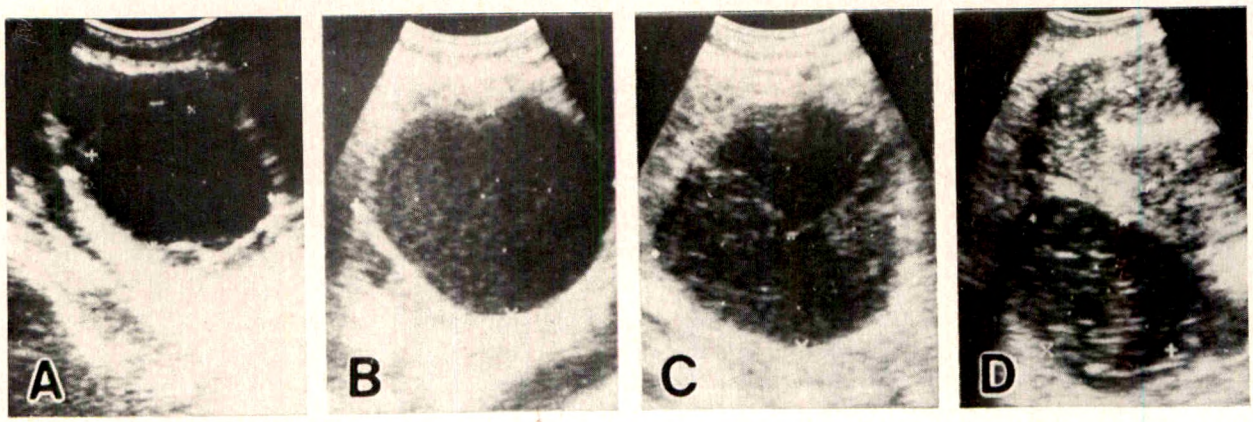


Fig. 1. Representative patterns of a luteinized unruptured follicle seen by transvaginal ultrasonography. **A,** Mature follicle (21-27 mm) on day 14 of menstrual cycle. **B,** Luteinized unruptured follicle (29-39 mm) on day 16 of the cycle. **C,** Enlarged luteinized unruptured follicle (36-37 mm) with intrafollicular echogenicity on day 18 of the cycle. **D,** Reduced size of luteinized unruptured follicle (30-36 mm) with increasing intrafollicular echogenicity on day 22 of the cycle.

Table I. Incidence of luteinized unruptured follicle

	% incidence of LUF (n)	
	In patients	*In cycles*
Endometriosis group	35 (11/31)*	25 (20/81)*
Control group	11 (3/28)	7 (3/44)

LUF, Luteinized unruptured follicle.
*$p < 0.05$ vs control.

Ultrasonographic documentation of LUF. Follicular development was monitored by transvaginal ultrasonography (SSD-650 and SSD-630; Aloka, Tokyo, Japan), daily or every other day, from day 8 of the menstrual cycle until 3 days after human chorionic gonadotropin administration. A diagnosis of luteinized unruptured follicle was established when the following signs were present: (1) a rise in basal body temperature; (2) ultrasonically persistent and enlarged follicles after human chorionic gonadotropin administration, without the collapse of the large follicle and free fluid in the pouch of Douglas (Fig. 1); (3) >5 ng/ml of serum progesterone during the mid luteal phase; and (4) a degenerated oocyte mass in the contents of persistent follicles.

Eighty-one cycles of 31 patients who had endometriosis were used to retrospectively investigate the occurrence of luteinized unruptured follicle during the year before laparoscopic examination. Forty-four cycles of 28 control patients who did not have endometriosis and whose husbands showed subnormal semen analysis were also analyzed for luteinized unruptured follicle. In luteinized unruptured follicle cycles follicular development and ovulation had been monitored by serial transvaginal ultrasonography. The concentration of serum progesterone during the mid luteal phase had been measured in most of the cycles. Furthermore, after the patients' informed consent was obtained, transvaginal, ultrasound-guided follicular aspiration of 14 follicles was performed as previously described[11] 3 to 5 days after human chorionic gonadotropin administration in 10 patients whose developed follicles did not rupture.

Results

Of 70 patients who were diagnosed with unexplained infertility, 47 (67%) had endometriosis; 40 (85%) of these had minimal ($n = 22$) or mild ($n = 18$) endometriosis. The remaining seven patients had moderate ($n = 5$) or severe ($n = 2$) endometriosis. Among the 23 women who did not have endometriosis, 2 had inflammatory adhesions of the pelvis and 21 had normal pelvic findings.

The incidence of luteinized unruptured follicle was 35% (11/31) among the patients and included 25% (20/81) of the cycles. In contrast, the incidence of this phenomenon in the control group was 11% (3/28) among the patients and included 7% (3/44) of the cycles monitored. The incidence of luteinized unruptured follicle among patients and the cycles monitored was significantly ($p < 0.05$) higher in the endometriosis group than in the control group (Table I).

Degenerated or postmature oocyte cumuli were observed in 6 (43%) of 14 luteinized unruptured follicles; the contents included yellowish-brown solids and a hazy and viscous fluid.

Comment

In this study we found that most of the patients who were diagnosed with unexplained infertility before laparoscopic examination had minimal or mild endome-

triosis. Moreover, the incidence of luteinized unruptured follicle was significantly higher in patients who had endometriosis than in those who did not have it.

Brosens et al.[3] postulated that luteinized unruptured follicle might be a cause of infertility in women who have endometriosis. Likewise, Donnez and Thomas[5] found that the incidence of luteinized unruptured follicle cycles was significantly higher in patients who had endometriosis (26% vs 9% in the control). Toda[6] recently reported that the incidence of luteinized unruptured follicle diagnosed by transvaginal ultrasonography was 13.5% per cycle monitored (n = 384) in patients who had infertility. A particularly higher incidence of luteinized unruptured follicle (range, 37.5% to 21.4%) was observed in patients who had polycystic ovarian syndrome, tubal infertility, and endometriosis when compared with the incidence in the case of male infertility and ovulatory disorder (range, 6.8% to 6.3%). The results in this study are comparable with the data reported in those articles. However, other investigators have reported very little association, if any, between luteinized unruptured follicle and endometriosis.[7-9]

When we consider these conflicting data, at least two aspects should be taken into account. One refers to the accuracy of the diagnosis of luteinized unruptured follicle by ultrasonography. Liukkonen et al.[12] reported that the diagnosis of the luteinized unruptured follicle syndrome by ultrasound examination seems to be fairly accurate according to the laparoscopic confirmation of the findings. However, Petsos et al.[13] emphasized that serial ultrasound scans alone cannot be used to detect ovulation. In this study transvaginal, ultrasound-guided follicular aspiration[11] of the luteinized unruptured follicle diagnosed by ultrasound examination was performed to examine the follicular contents during the mid luteal phase. Forty-three percent (6/14) of the luteinized unruptured follicles contained degenerated oocyte masses in the mid luteal phase. The results demonstrate that this technique may be useful to establish a definitive diagnosis of luteinized unruptured follicle and to elucidate the mechanisms involved in this phenomenon.

We must also consider how minimal or early endometriosis interferes with the process of ovulation and fertilization. It is theorized that prostaglandins,[14] proteolytic enzymes,[15] and cellular components such as macrophages and lymphocytes[16] in the peritoneal fluid of patients who have minimal or mild endometriosis may individually or jointly affect the ovulation. In addition, it is known that the peritoneal fluid itself is toxic to the growth and development of embryos in the early stages of embryogenesis.[1,16] Therefore minimal or mild endometriosis may be associated with infertility. However, the relationship between luteinized unruptured follicle and endometriosis-associated infertility remains to be elucidated.

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Luteal function in infertile patients with endometriosis

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OBJECTIVE: Our purpose was to determine whether infertile patients who have endometriosis show luteal phase defects.
STUDY DESIGN: The luteal function in 24 infertile patients who had endometriosis was compared with the luteal function in 20 patients who had unexplained infertility and did not have endometriosis (control). In both groups serum luteinizing hormone, follicle-stimulating hormone, progesterone, and estradiol were assayed every day throughout the menstrual cycle. Endometrial biopsy specimens were obtained from eight patients of the endometriosis group for histologic dating of the endometrium.
RESULTS: No significant differences in progesterone levels were observed between these two groups during the mid and late luteal phase. Seven of the eight patients who underwent histologic dating showed a luteal phase pattern, whereas only one patient was out of phase.
CONCLUSION: Infertile patients who have endometriosis do not always have luteal phase defects. (Am J Obstet Gynecol 1992;167:274-7.)

Key words: Endometriosis, luteal function, infertility

The real cause of infertility in patients who have endometriosis has not yet been clarified. Functional or anatomic abnormalities of reproductive organs within the pelvic cavity that are induced by extended endometriosis are considered to be a direct cause of infertility; however, the presence of organ abnormalities alone does not explain the cause of infertility in patients who have mild and moderate endometriosis. There must be other factors associated with endometriosis that cause infertility in these patients. One of the possible causes is ovarian dysfunction. Luteal phase defects in particular have been suggested as a plausible explanation. Williams et al.[1] reported that the progesterone level is low in patients who have endometriosis. Conversely, Radwanska and Dmowski[2] reported that endometriosis is not complicated by luteal phase defects. Thus no conclusions have been reached regarding the presence or absence of luteal phase defects in infertile patients who have endometriosis.

Consequently, in the current study I compared the progesterone levels of infertile patients who had endometriosis and patients who had unexplained infertility without endometriosis to clarify whether infertile patients with endometriosis have luteal phase defects.

Material and methods

The subjects were 20 patients who had unexplained infertility in whom the absence of endometriosis was confirmed by laparoscopic examination (control group) and 24 infertile patients who had laparoscopically confirmed endometriosis (endometriosis group). No significant differences in age and the duration of infertility existed between these groups (Table I).

Endometrial biopsy specimens were obtained from eight patients of the endometriosis group 7 days after the luteinizing hormone (LH) surge and were histologically examined. Dating was conducted according to the criteria proposed by Noyes et al.[3]

Blood samples were taken every day throughout the menstrual cycle from each of the patients ($n = 44$), and serum was stored until assayed to determine the concentrations of LH, follicle-stimulating hormone, estradiol, and progesterone in the course of time.

A kit for radioimmunoassay from Daiichi Radio Isotope Inc. (Tokyo, Japan) was used to measure serum LH, follicle-stimulating hormone, estradiol, and progesterone. The coefficient value was satisfactory; it was 5% to 9% or less in the intraassay and 8% to 14% in the interassay. Statistical significance was analyzed by the Student t test.

Results

Fig. 1 demonstrates the results of the comparison of gonadotropins levels between the control group (*shadow*, mean values ± SD) and the group of patients who had endometriosis (*open circle*, mean values ± SD). The date of the LH surge was designated as day 0. The value of the LH surge in the control group was 91.2 ± 50.0 IU/ml. Similarly, the value of the LH surge in the endometriosis group was 105.5 ± 55.5 IU/ml, which revealed no significant differences between these two groups.

Moreover, comparison of the basal LH levels in the follicular and luteal phases in the control and endometriosis groups revealed no significant differences.

Follicle-stimulating hormone levels were also com-

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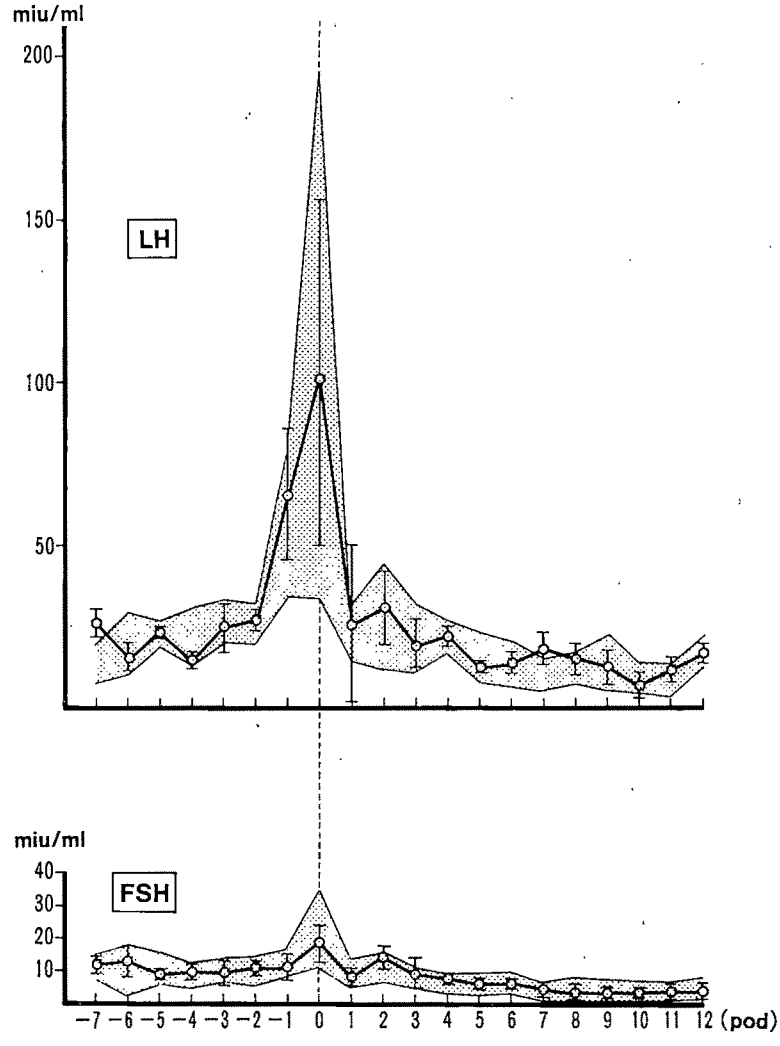


Fig. 1. Serum LH and follicle-stimulating hormone concentrations in control (*shaded background, n* = 20) and endometriosis (*open circle, n* = 24) groups. Data are expressed as mean values ± SD.

pared between the control and endometriosis groups (Fig. 1). No significant differences were observed between the two groups regarding the basal follicle-stimulating hormone levels in the follicular or luteal phase.

Progesterone and estradiol levels in both groups were compared to investigate the luteal function in the endometriosis group. Fig. 2 shows the changes in the levels of progesterone and estradiol in the control and endometriosis groups. The progesterone level in the control group rose immediately after ovulation, reached the peak value of 16.3 ± 4.7 ng/ml on day +6, and then declined. On the other hand, in the endometriosis group the level was slightly low on days +1 and +2, but it reached the peak value of 14.1 ± 5.5 ng/ml on day +7. The peak value was not significantly different from the peak value in the control group. No significant differences from the control group were observed even during the subsequent late luteal phase.

Table I. Characteristics of the study population

	Endometriosis group (n = 24)	*Control group (n = 20)*
Age (yr)	30.7 ± 4.3	31.5 ± 5.3
Duration of infertility (yr)	4.3 ± 3.2	3.7 ± 2.5
Endometriosis stage*	Minimal 2 cases Mild 10 cases Moderate 6 cases Severe 6 cases	—

Data are expressed as mean values ± SD.
*Revised American Fertility Society Classification.

Regarding estradiol levels, the preovulatory surge in the control group occurred on day −1 (237.6 ± 78.9 pg/ml) and the mid luteal surge occurred on day +7 (176.7 ± 60.8 pg/ml). In contrast, although estradiol

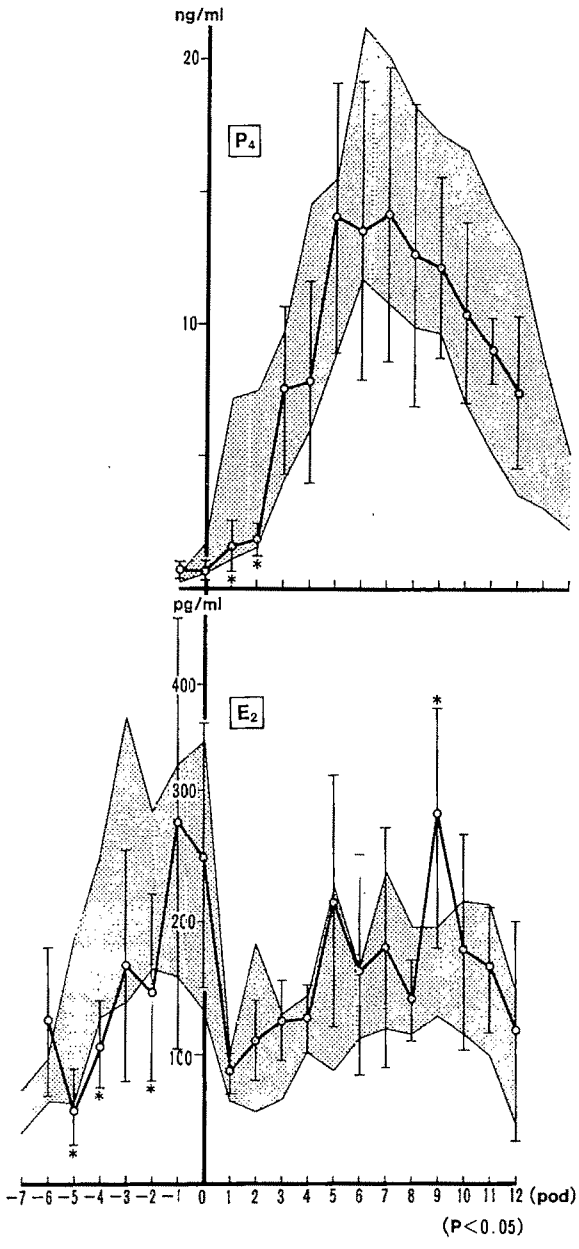


Fig. 2. Serum progesterone and estradiol concentrations measured during luteal phase in control (*shaded background, n* = 20) and endometriosis (*open circle, n* = 24) groups. Data are expressed as mean values ± SD.

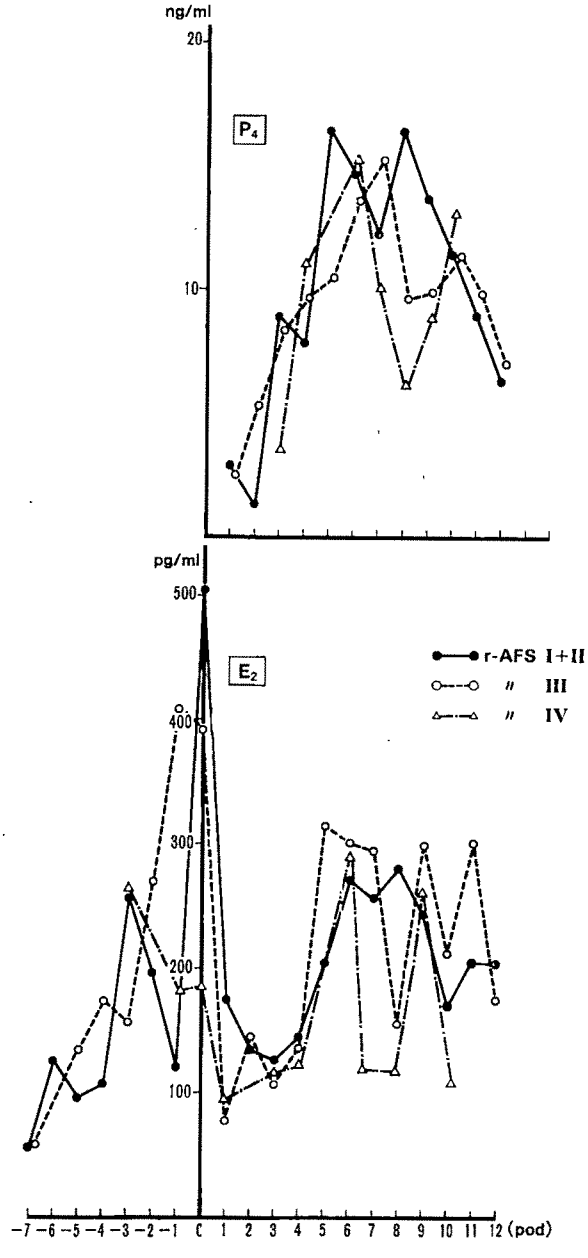


Fig. 3. Mean value of progesterone and estradiol in patients who had endometriosis classified according to stage of disease. Stage I + II (*closed circle, n* = 12), stage III (*open circle, n* = 6), and stage IV (*open triangle, n* = 6). Data are expressed as mean values.

levels in the endometriosis group were slightly lower during the late follicular phase, no significant differences in the control group were observed. In the endometriosis group the estradiol levels reached the preovulatory peak of 277.8 ± 174.5 pg/ml on day −1, and the mid luteal surge of 215.7 ± 95.0 pg/ml occurred on day +5.

The endometriosis group was then divided into three groups according to the stage of the disease on the basis of the revised American Fertility Society Classification: the first group consisted of patients who had stage I and II disease, the second group consisted of patients who had stage III disease, and the third group consisted of those who had stage IV disease. The progesterone and estradiol levels in these three groups are shown in Fig. 3.

Although patients who had stage IV disease showed slightly lower progesterone and estradiol levels on day +8, no significant differences were observed regarding these two parameters when compared with those pa-

tients who had stage I, II, or III disease. A decline in progesterone levels along with the progression of endometriosis was not observed.

Finally, histologic dating of endometrial tissues performed in 8 of the 24 patients who had endometriosis demonstrated that 7 of the 8 patients showed a luteal phase pattern, whereas only 1 patient was out of phase.

Comment

It has been noted that endometriosis is accompanied by mild ovarian dysfunctions such as anovulation[4] and hyperprolactinemia,[5] but luteal phase defects are said to be one of the most typical ovarian dysfunctions in patients who have endometriosis-associated infertility.[6]

Methods for the evaluation of the luteal function include use of a basal body temperature chart and endometrial biopsies. In the current study progesterone levels in patients who had endometriosis-associated infertility were compared with those of patients who had unexplained infertility without endometriosis, because all of the conflicting results in the literature[1, 2, 6] were in relation to progesterone function.

Hargrove and Abraham[7] reported for the first time that because of the low progesterone levels in infertile patients who have endometriosis during the mid luteal phase, abnormal luteal function is often observed in these patients. Moreover, Williams et al.[1] continuously measured the progesterone levels throughout the luteal phase and also pointed out that luteal phase defects often occur in these patients because progesterone levels are significantly lower during the early and mid luteal phases.

On the other hand, numerous reports state that luteal phase defects do not occur so frequently in infertile patients who have endometriosis. Radwanska and Dmowski[2] compared the mid luteal serum levels of progesterone and the preovulatory serum levels of estradiol in clinically ovulatory women and infertile patients who had endometriosis, and they observed no significant differences between the nonendometriosis and endometriosis groups regarding the levels of progesterone and estradiol. In light of these findings Randwanska and Dmowski[2] concluded that luteal deficiency is not more likely to occur in patients who have endometriosis and that luteal deficiency is not the direct cause of endometriosis-associated infertility. Furthermore, Pittaway et al.[8] evaluated the luteal function in endometrial biopsies and reported that the incidence of luteal phase defects was 5.3% in the nonendometriosis group and 8.8% in the endometriosis group, which demonstrates that no significant difference exists between these groups.

In the current study progesterone levels during the luteal phase were measured in patients who did and did not have endometriosis. The results contradicted the previous results reported by Cheesman et al.[6]; that is, the progesterone levels of the patients evaluated in this study were slightly lower during the early luteal phase (days +1 and +2) but were completely normal during the mid and late luteal phases. Besides, no correlation existed between the stage of endometriosis and progesterone levels.

Moreover, histologic dating of the endometrium during the mid luteal phase revealed that the endometrium was abnormally retarded in only one of eight patients (12.5%).

In short, although progesterone levels were low in the endometriosis group during the early luteal phase (days +1 and +2), they returned to normal soon afterward. Consequently, it would be difficult to regard luteal phase defects as the main cause of endometriosis-associated infertility.

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The impact of endometriosis on the reproductive outcome of infertile patients

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OBJECTIVE: We examined whether the presence and severity of endometriosis affect the reproductive outcome of infertile patients.

STUDY DESIGN: The conception rates of 2080 infertile women, 1263 who had endometriosis and 817 who did not have endometriosis, were analyzed retrospectively by means of the χ^2 test. Depending on the stage of the disease patients who had endometriosis-associated infertility underwent expectant management, danazol therapy, or minor or major conservative surgery. The patients who failed to conceive after these conventional treatments were enrolled in the in vitro fertilization–embryo transfer program.

RESULTS: The conception rates were virtually identical regardless of the presence or absence of endometriosis (30.7% vs 30.0%). The outcome of in vitro fertilization–embryo transfer was not affected either by the presence or the severity of the disease.

CONCLUSION: Endometriosis had no impact on the reproductive outcome of infertile patients in this series unless the anatomy of the pelvic organs was heavily distorted, which can occur in the advanced stages of the disease. (Am J Obstet Gynecol 1992;167:278-82.)

Key words: Endometriosis, infertility, electrocoagulation, danazol, in vitro fertilization–embryo transfer

Minimal and mild endometriosis is frequently found in infertile women,[1] and a definite decrement of fertility has been reported even in patients who have minimal endometriosis.[2] Many possible causes have been proposed to explain the relationship between endometriosis and infertility.[3, 4] More recently, after the advent of in vitro fertilization–embryo transfer (IVF-ET), it has been implied that endometriosis affects the quality of oocytes; therefore the fertilization rates are low.[5] However, the exact mechanism by which minimal endometriosis interferes with fertility is still unknown.[3, 4]

Various types of therapy have been devised to treat endometriosis-associated infertility; these include expectant management,[1, 6] medical treatment,[7, 8] conservative surgery,[2, 9] and operative laparoscopy.[10, 11] Nevertheless, no medical or surgical therapy has been shown to be truly efficacious.[12]

It has recently been reported that the fertility rate is normal in women who have minimal endometriosis,[13] which casts doubt on the relationship between endometriosis and infertility.

In this study we analyzed our data retrospectively to determine whether the presence and severity of endometriosis affect the reproductive outcome of infertile patients. The cause-and-effect relationship between endometriosis and infertility is also discussed.

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Material and methods

By the end of December 1988, 2080 women had undergone diagnostic laparoscopic examination at our hospital as part of an infertility evaluation. Their mean age was 32.3 years, and the mean infertility period was 6.0 years. Of the entire study population 1263 (60.7%) patients had endometriosis.

Endometriosis was confirmed by direct laparoscopic visualization or biopsy of lesions. The severity of disease was determined according to the American Fertility Society revised classification. The laparoscopic procedures usually included ascites aspiration from the pouch of Douglas, chromopertubation, and cervical dilation and curettage.

Treatment of stage I and II endometriosis. At first most patients (n = 359) who had stage I and II disease underwent expectant management of infertility for 1 year after laparoscopy. During this period all other major infertility factors were corrected if found. Those who did not conceive then started therapy with danazol (600 mg/day for 6 months) or were treated by laparoscopic electrocoagulation.

The policy was later changed, and patients (n = 442) who had minimal or mild disease were treated exclusively by electrocoagulation at the time of the initial

Table I. Conception rates of infertile patients with and without endometriosis

Endometriosis	*No. of patients*	*Mean age (yr)*	*Mean infertility period (yr)*	*No. of women who conceived*	*%*
(+)	1263	32.5	6.0	388	30.7*
(−)	817	31.9	6.0	245	30.0
Total	2080	32.3	6.0	633	30.4

Pregnancies after IVF of gamete intrafallopian transfer were excluded.
*Not significantly different from conception rates in women without endometriosis.

Table II. Stage of endometriosis and conception rates

Stage	*No. of patients*	*Mean age (yr)*	*Mean infertility period (yr)*	*No. of women who conceived*	*%*
I	587	32.3	6.0	204*	34.8
II	348	32.5	6.0	100	28.7
III	184	32.6	5.7	53	28.8
IV	144	33.1	6.4	31*	21.5
Total	1263	32.5	6.0	388	30.7

Pregnancies after IVF or gamete intrafallopian transfer were excluded.
*$p < 0.005$.

laparoscopy. A monopolar cautery was applied to the endometriotic implants until the lesions and the surrounding tissues blanched. Adhesions were lysed sharply with a monopolar needle-point cautery.

After electrocoagulation the pelvis was irrigated with saline solution. No complications during or after the operation were noted among these patients.

Treatment of stage III and IV endometriosis. At initial laparoscopic examination, ovarian endometriomas were aspirated and irrigated with saline solution. Adhesions around the ovaries and tubes, when found, were lysed sharply with monopolar microelectrodes. They then underwent expectant management for infertility for 4 to 6 months. The patients who did not conceive were prescribed danazol (600 mg/day) for 6 months and were followed up for at least 1 year. Recurrent endometriomas were managed laparoscopically or were aspirated through the vagina under ultrasonographic control. Major conservative surgery ($n = 41$) was performed only when other infertility factors, such as myomas or hydrosalpinx, were found.

Treatment of patients who did not have endometriosis. These patients were treated individually according to the infertility factors: tubal factors were corrected laparoscopically or by microsurgery, myomas were enucleated when indicated, and patients who had a normal pelvis were subjected to empiric treatment, such as ovarian stimulation with or without intrauterine insemination.

In vitro fertilization and embryo transfer. A total of 309 patients who had endometriosis and 392 patients who did not have endometriosis and who did not conceive after conventional treatments (expectant management, danazol, and electrocoagulation) were enrolled in the IVF-ET program. Their mean age was 34.9 years and 33.3 years, respectively, and the mean duration of infertility was 8.8 years and 8.5 years, respectively.

Ovarian stimulation was accomplished with a combination of clomiphene citrate and human menopausal gonadotropin or human menopausal gonadotropin alone, with or without gonadotropin-releasing hormone agonists.

The response to treatment was monitored by ultrasound studies together with the measurement of serum estradiol. Ovulation was triggered by the administration of 10,000 IU of human chorionic gonadotropin when at least two follicles greater than 17 mm in diameter were present and the serum estradiol level was between 200 and 300 pg/ml per mature follicle. Follicular aspiration by laparoscopy or transvaginal ultrasound guidance was performed 36 hours after human chorionic gonadotropin injection.

Semen was obtained by masturbation, and motile spermatozoa were prepared by the standard swim-up procedure. Oocytes were inseminated (1×10^5 motile sperm/ml) 4 to 8 hours after collection, pronuclei were identified 16 to 20 hours later, and ET was performed 24 to 72 hours after insemination. The media used were Ham-F10 or human tubal fluid supplemented with 7.5% (insemination medium) and 15% (growth medium) heat-inactivated human cord serum.

All patients who underwent ET received supplemental progesterone throughout the luteal phase. Pregnancy was diagnosed when a highly-sensitive pregnancy test (Testpack, Dainabot Co., Ltd., Tokyo,

Table III. Conception rates after expectant management of endometriosis

Endometriosis	*No. of patients*	*No. of women who conceived*	*%*
Stage I	241	87	36.1
Stage II	118	35	29.7

Table IV. Conception rates after undergoing electrocoagulation treatment for endometriosis

Endometriosis	*No. of patients*	*No. of women who conceived*	*%*
Stage I	276	86	31.2
Stage II	166	36	21.7

Japan), which was performed 14 to 16 days after ET was positive. Statistical analysis of the data was performed with the χ^2 test.

Results

The mean age and mean infertility period of patients who had endometriosis (32.5 years and 6.0 years) and who did not have endometriosis (31.9 years and 6.0 years) were not statistically different. Interestingly, these two parameters were also almost identical when the patients were compared according to the stage of endometriosis.

By the end of August 1991, 633 patients (30.4%) had conceived after conventional therapy that included intrauterine insemination. The presence of endometriosis did not affect the conception rate of infertile patients (Table I). The conception rate slightly declined as the stage of the disease progressed (Table II). However, a statistically significant difference was observed only between stage I and stage IV ($p < 0.005$). A considerable number of patients who had stage IV endometriosis had undergone major conservative surgery before coming to our hospital, and laparoscopic examination revealed that many of them had severely distorted pelvic organs. Therefore the difference between stage I and stage IV in terms of conception rate should be interpreted with caution. To address the cause-and-effect relationship between endometriosis and infertility, expectant management and laparoscopic electrocoagulation were compared in stage I and stage II disease. A total of 241 stage I patients and 118 stage II patients underwent expectant management for at least 1 year after laparoscopy. The conception rates were almost satisfactory (Table III). Ninety-eight patients who did not conceive after expectant management received therapy with danazol (600 mg/day for 6 months). However, the results were very disappointing. Of 44 patients who had stage I disease, only 3 (6.8%) became pregnant, and none of 54 stage II patients conceived.

Unexpectedly, electrocoagulation did not increase the pregnancy rate. The patients whose endometrial implants were treated by electrocoagulation showed somewhat lower pregnancy rates compared with patients who underwent expectant management (Table IV). The results of IVF-ET are given in Tables V and VI. The number of oocytes that were retrieved and fertilized and the number of embryos transferred were almost the same, regardless of the presence or absence of endometriosis. Moreover, the presence of endometriosis did not affect the conception rate of the IVF-ET program.

The patients who had endometriosis had a somewhat higher pregnancy rate per ET compared with those who did not have endometriosis. Furthermore, the severity of the disease did not affect the pregnancy rate either per patient or per ET.

Comment

A strong association exists between infertility and endometriosis. In this study we found endometriosis in 60.7% of the infertile patients. Koninckx et al.[14] have also recently reported a 68% incidence of endometriosis in patients who underwent laparoscopy for infertility. Interestingly, the mean age of our patients and their mean infertility period were almost identical, regardless of the presence or absence of endometriosis or the stage of the disease. The natural history of endometriosis is poorly understood. Mild endometriosis can progress if left untreated.[15,16] It is also true, however, that endometriotic implants can either regress spontaneously or not progress when left untreated.[16] The present data support the previous observation that endometriosis may not always progress from stage I to stage II and eventually to stage IV.[16]

Patients who have minimal disease and those who have severe disease may represent two different spectrums of this disease, and different mechanisms may be involved in infertility, as suggested by Mahmood and Templeton.[16]

Endometriosis has been implicated as a cause of infertility. Acosta et al.[2] reported a definite reduction in fertility even in patients who had mild endometriosis. Several hypotheses have been proposed to explain the relationship between endometriosis and infertility; these include altered prostaglandin secretion and metabolism, increased macrophage activity, ovarian dysfunction, and aberrant immune response.[3,4] In recent years, however, doubt has been cast on the causal relationship between endometriosis and infertility. Rodriguez-Escudero et al.[13] have demonstrated that in patients who have minimal endometriosis the fertility and

Table V. Results of IVG-ET in patients with and without endometriosis

Endometriosis	*No. of patients*	*No. of ET*	*No. of patients who conceived*	*No. of conceptions*	*Conception rate*	
					Per patient	*Per ET*
(+)	309	476	131	147	42.4*	30.9*
(−)	372	701	165	189	44.4	27.0
Total	681	1177	296	336	43.5	28.5

*Not significantly different from conception rates in women without endometriosis.

Table VI. Results of IVF-ET in relation to the stage of endometriosis

Stage	*No. of patients*	*No. of ET*	*No. of patients who conceived*	*No. of conceptions*	*Conception rate*	
					Per patient	*Per ET*
I	111	171	45	52	40.5	30.4
II	78	123	33	39	42.3	31.7
III	51	76	24	24	47.1	31.6
IV	69	106	29	32	42.0	30.2
Total	309	476	131	147	42.4	30.9

monthly fecundity rates are normal on the basis of their experience with artificial insemination by means of donor semen.

Indeed, endometriosis is not infrequent in fertile women. Moen[17] reported a group of 108 women who underwent laparoscopic sterilization; endometriosis was found in 18% of these patients. Liu and Hitchcock[18] noted a high incidence of endometriosis (43%) in a group of 75 patients who sought laparoscopic sterilization. Furthermore, expectant management or no treatment, interestingly enough, yields pregnancy rates that are similar to the outcome of medical or surgical intervention.[12, 19] Thus endometriosis of minimal or mild degree may be an incidental finding and of no importance in the pathogenesis of infertility.

The results of this study also question the cause-and-effect relationship between endometriosis and infertility. The presence of endometriosis did not affect the crude pregnancy rates of infertile patients. Moreover, no significant difference in pregnancy rates was found between those who underwent expectant management and those who underwent laparoscopic electrocoagulation; when the patients who had stage I and stage II disease were compared, elimination of endometrial implants did not result in an improvement of fecundity. The presence of endometriosis may be a marker of some other acquired or developmental pathologic state that may be the cause of infertility and that remains after the endometriosis is eradicated, as suggested by Redwine.[20]

The severity of endometriosis did not affect the reproductive success rate of infertile patients. No significant difference was found between patients who had stage I, stage II, and stage III disease when the crude pregnancy rates were compared.

A statistically significant difference in conception frequency was noted only between stage I and stage IV. However, it should be remembered that a considerable number of patients in stage IV had received major conservative surgery before they were referred to our hospital, and many of them revealed heavily distorted pelvic organs at laparoscopy; therefore, conventional treatments were ineffective for the treatment of fertility.

The predominant feature of stage III and stage IV disease was the presence of ovarian endometriomas, which were resistant to treatment and visualized by vaginal ultrasound scans in most patients even after pregnancy was established.

The advent of IVF-ET has provided a unique opportunity to assess the potential effect of endometriosis on oocyte quality or the implantation process. Earlier studies have shown that endometriosis affects the pregnancy rate of IVF-ET programs.[5, 21-23]

Wardle et al.[5] have reported that mild or moderate endometriosis affects the fertilization rate by reducing the quality of oocytes compared with that of women who have tubal disease only. Others have shown that the fecundity rate per cycle in women who had stage I and stage II endometriosis was similar to that of the group with tubal factors, but it was significantly reduced in women who had stage IV disease.[21, 22] A reduced number of recovered oocytes and a decreased implantation rate per embryo transferred might be responsible for the reduced pregnancy rate in patients who have severe endometriosis after IVF-ET, which sug-

gests the presence of an implantation inhibitory factor in these patients.[23]

More recently, however, Garcia et al.[24] and Gerday et al.[25] have reported that severity of endometriosis does not impair the follicular recruitment parameters, the oocyte fertilizing ability, or the pregnancy rate. Our results support these recent observations. The presence of endometriosis did not affect the pregnancy rate after IVF-ET, and the severity of the disease did not affect the pregnancy rate either per patient or per embryo transfer.

It appears unlikely that endometriosis affects the quality of oocytes or impairs embryo implantation. The patients who had endometriosis and did not conceive after conventional therapy should be treated by IVF-ET irrespective of the stage of the disease.

In conclusion, the reproductive outcome of infertile patients was not significantly affected either by the presence of endometriosis or by the stage of the disease unless the pelvic organs were heavily distorted because of previous conservative surgery. Furthermore, neither the presence nor the severity of the disease affected the outcome of IVF-ET.

These results cast serious doubt on a cause-and-effect relationship between endometriosis and infertility. Endometriosis may be an incidental finding and of no importance in the pathogenesis of infertility except for the terminal stage, in which the anatomy of pelvic organs is heavily distorted.

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Mechanisms involved in the pituitary desensitization induced by gonadotropin-releasing hormone agonists

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OBJECTIVE: We investigated the mechanisms of desensitization induced by gonadotropin-releasing hormone agonist in the pituitary.
STUDY DESIGN: Effects of gonadotropin-releasing hormone agonist on the pituitary were studied in vitro and in vivo in the rat. In the clinical study serum luteinizing hormone was measured by radioimmunoassay with a polyclonal luteinizing hormone antibody (luteinizing hormone-radioimmunoassay) and by immunoradiometric assay with monoclonal luteinizing hormone antibodies (luteinizing hormone-immunoradiometric assay) during gonadotropin-releasing hormone agonist treatment.
RESULTS: In the in vitro study bead-attached pituitary cells that were desensitized with a continuous infusion of 10^{-7}mol/L gonadotropin-releasing hormone responded to 50 mmol/L K^+. In the in vivo study gonadotropin-releasing hormone binding sites and rat luteinizing hormone β–messenger ribonucleic acid in the pituitary decreased during gonadotropin-releasing hormone agonist treatment, but serum levels of rat luteinizing hormone did not decrease. In addition, a disparity between luteinizing hormone-radioimmunoassay and luteinizing hormone-immunoradiometric assay was demonstrated during gonadotropin-releasing hormone agonist treatment.
CONCLUSION: Pituitary desensitization in response to gonadotropin-releasing hormone agonist may not be wholly receptor mediated and a nonreceptor process may be involved. (Am J Obstet Gynecol 1992;167:283-91.)

Key words: Pituitary desensitization, gonadotropin-releasing hormone agonist, luteinizing hormone, luteinizing hormone β–messenger ribonucleic acid, gonadotropin-releasing hormone receptor

Gonadotropin-releasing hormone agonists (GnRHa) have proved useful for the treatment of endometriosis because GnRHa affect only specific organs and induce hypogonadotropic hypoestrogenism (desensitization), which renders the endometrial implant atrophic. However, the mechanisms involved in the desensitization of the pituitary are still poorly understood.

To investigate these mechanisms, we administered GnRH agonist to rats and humans for more than 4 weeks. We then measured the concentrations of serum rat luteinizing hormone (LH) and pituitary rat LH, the response of serum rat LH to GnRH, and GnRH-binding sites and gene expression of rat LH β in the pituitary in the rat. In humans who were undergoing GnRH agonist treatment, we investigated the concentration of serum LH determined by a polyclonal antibody and by monoclonal antibodies. We also examined rat pituitary cells in vitro.

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6/0/38234

Material and methods

Desensitization of pituitary cells in vitro. Pituitary cells obtained from Wistar male rats were dispersed in HEPES dissociating buffer (137 mmol/L sodium chloride, 5 mmol/L potassium chloride, 0.7 mmol/L disodium acid phosphate, and 25 mmol/L HEPES, pH 7.3) with the use of 0.4% of collagenase type II and 10 μg/ml deoxyribonuclease II (Sigma Chemical Co., St. Louis, Mo.). The final cell suspension (approximately 10^6 cells/ml) was cultured with swollen and autoclaved Cytodex beads (Pharmacia Biosystems, Piscataway, N.J.) at a concentration of 0.1 ml beads/ml of cell suspension as described by Smith and Vale.[1] The bead-attached cells were packed into mini-columns (0.6 × 10 cm, Bio-Rad Laboratories, Richmond, Calif.) and superfused at a constant flow rate (0.13 ml/min). The cells were first superfused with GnRH and then with the test substances; effluent fractions were collected at 6-minute intervals and stored at −20° C until assayed.

Study of rats in vivo

Animals and sample collection. Immature Sprague-Dawley female rats (21 days of age) were administered a sustained-release formulation of GnRH agonist (TAP-144-SR; D-Leu^6-[des-Gly^{10}-NH_2] GnRH-EA, Takeda

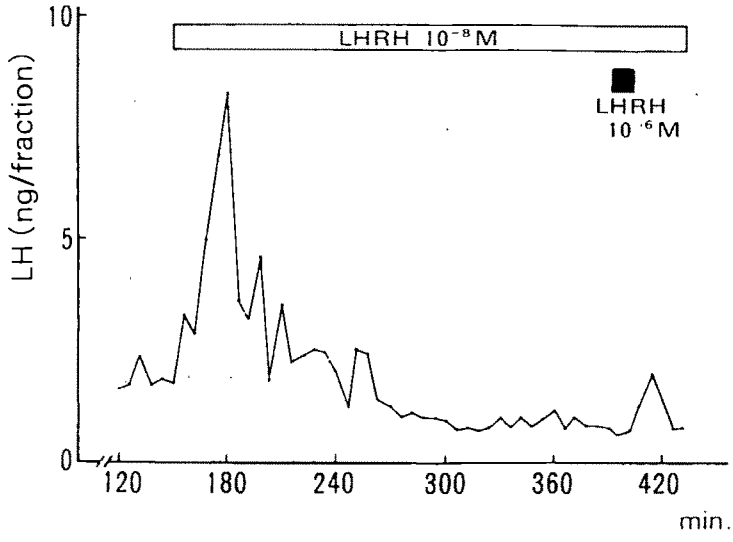


Fig. 1. Rat LH responses of bead-attached pituitary cells to 10^{-6} mol/L GnRH after continuous infusion of 10^{-8} mol/L GnRH.

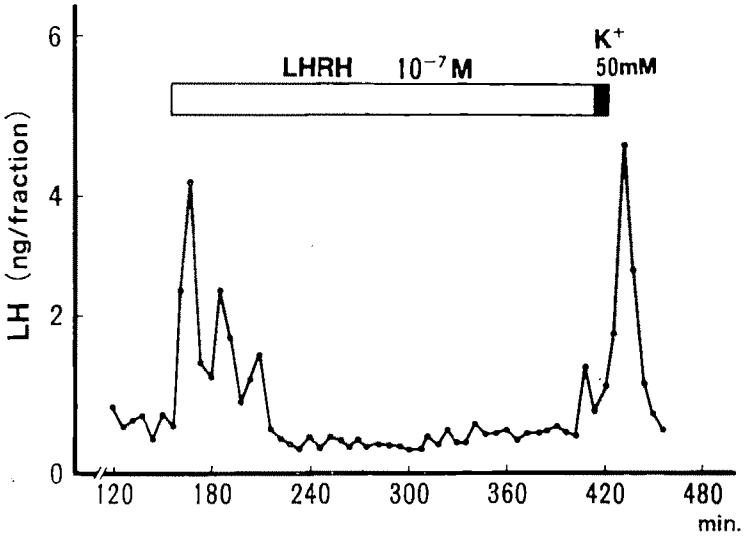


Fig. 2. Rat LH responses of bead-attached pituitary cells to 50 mmol/L K^+ after continuous infusion of 10^{-7} mol/L GnRH.

Chemical Industries, Ltd., Osaka, Japan) subcutaneously at a dose of 0.94 mg per rat every 4 weeks and were killed by decapitation at various time intervals after GnRH agonist injection. The pituitaries were harvested under sterile conditions and stored at −70° C until subsequent analysis. Trunk blood was collected and stored at −20° C until assayed.

To monitor the pituitary response to GnRH blood samples were obtained by heart puncture under light ether anesthesia before and 30 minutes after a subcutaneous injection of 1 μg of a native GnRH (Lutamin, Daiichi Medical Co., Ltd., Tokyo, Japan) on days 0, 7, 14, 21, and 28.

GnRH receptor assay. With use of a GnRH agonist (buserelin; [D-Ser (tBu^6) des-gly-NH_2^{10}] GnRH-ethlamide, Hoechst Japan Co., Ltd., Tokyo, Japan) as a ligand, the binding capacity of the rat pituitary was examined as described previously.[2]

Assay of rat LH β-messenger ribonucleic acid. The preparation of rat pituitary messenger ribonucleic acid (mRNA) for assay by dot blot hybridization was performed as described by Abbot et al.[3] Briefly, the pitui-

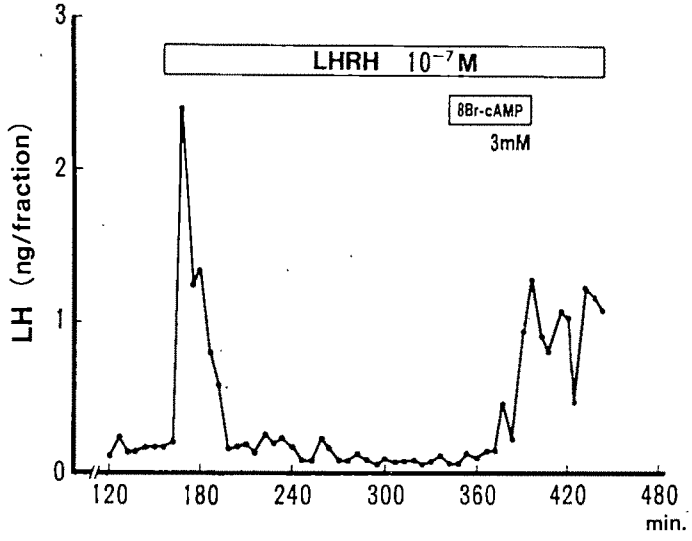


Fig. 3. Rat LH responses of bead-attached pituitary cells to 8-bromo-cyclic adenosine monophosphate after continuous infusion of 10^{-7} mol/L GnRH.

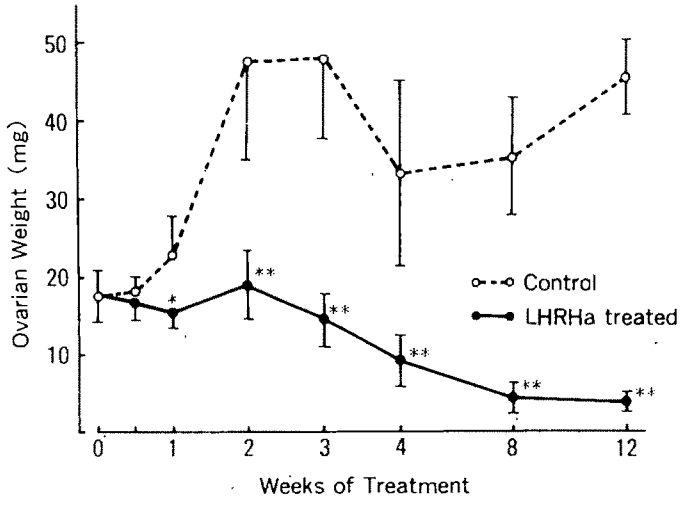


Fig. 4. Changes in ovarian weight during GnRHa treatment (0.94 mg/4 weeks) (mean ± SE, $n = 5$). $^{*}p < 0.05$, $^{**}p < 0.01$ compared with controls.

taries were homogenized in 200 μl of homogenization buffer and centrifuged at 15,000*g*. Proteins in the supernatant were denatured, extracted with phenol, and reextracted with chloroform. The RNA was precipitated overnight with ethanol and 3 mol/L sodium acetate at −70° C. The RNA was recovered by centrifugation at 15,000*g* for 30 minutes and dissolved in sterile distilled water.

A synthetic labeled oligodeoxynucleotide was produced as described by Gharib et al.[4] RNA samples of 5 μg each were applied to a gene hybridization membrane, which was baked at 80° C for 2 hours before prehybridization. The baked membrane was incubated at 65° C for 4 hours with 5 ml of prehybridization buffer before incubation with 50 to 100 ng of (phosphorus 32) complementary deoxyribonucleic acid for 3 hours at 60° C. After hybridization excess unbound (phosphorus 32) complementary deoxyribonucleic acid was removed by sequential washing with constant agitation in buffer. After washing, the membrane was dried, placed in contact with an X-ray film and autoradiographed for 16 to 24 hours at −70° C. The mRNA

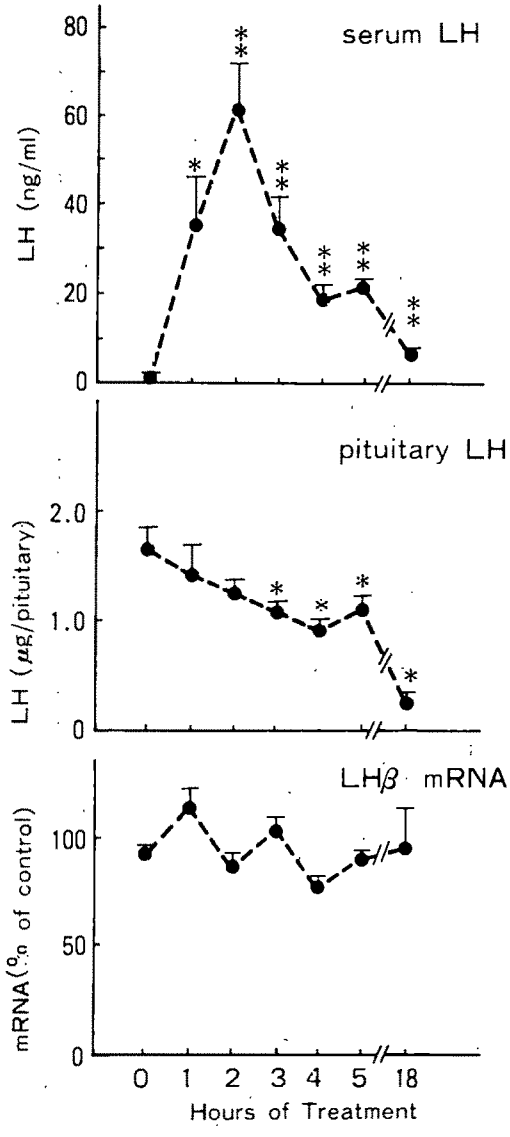


Fig. 5. Serum rat LH (mean ± SE, $n = 5$), pituitary rat LH content ($n = 5$) and rat LH β-mRNA ($n = 3$ to 4) from 0 to 18 hours after GnRH agonist treatment. Levels of rat LH β-mRNA are expressed as a percentage of the control. $*p < 0.05$, $**p < 0.01$ compared with the control value (hour 0).

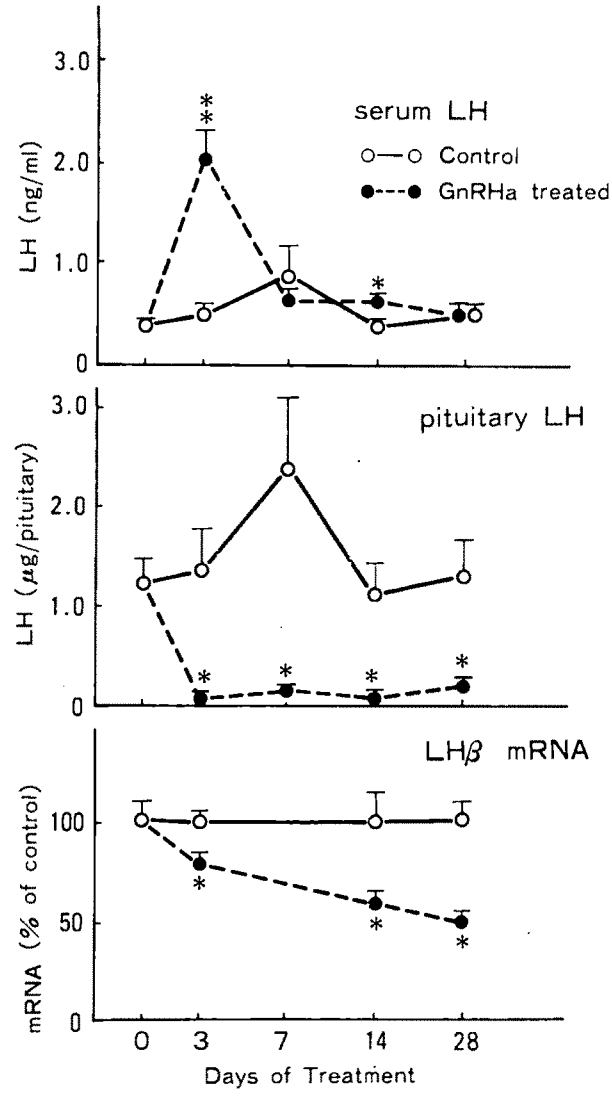


Fig. 6. Serum rat LH (mean ± SE, $n = 5$), pituitary rat LH content ($n = 5$), and rat LH β-mRNA ($n = 5$) from 0 to 28 hours after GnRH agonist treatment. The levels of rat LH β-mRNA are expressed as a percentage of the controls. $*p < 0.05$, $**p < 0.01$ compared with controls.

content of each dot was determined by densitometry of autoradiograms with a densitometer (Ultrascan XL, Pharmacia LKB Nuclear, Gaithersburg, M.D.).

Clinical study. A GnRH agonist (nafarelin; [D-Ala-3(2-naphtyl)^6] GnRH, Nippon Syntex KK, Tokyo, Japan) was administered intranasally at a dose of 400 μg/day for 3 weeks to eight patients who had polycystic ovarian disease and seven infertile patients. Another GnRH agonist (TAP-144-SR) was administered subcutaneously at a dose of 3.8 mg per 4 weeks for 24 weeks to 16 eumenorrheic patients who had endometriosis. Blood samples were obtained twice a week, either every week or every 2 weeks. Serum levels of LH were examined by radioimmunoassay (RIA) with a polyclonal antibody and by immunoradiometric assay (IRMA) with monoclonal antibodies.

Hormone assays and statistical analyses. Serum and pituitary homogenates were assayed for rat LH by a double-antibody RIA technique with rat hormone kits supplied by the National Pituitary Agency (NIDDK, USA), and rat LH concentrations were expressed in NIH-LH-SI. "Daiichi" LH-kit (Daiichi Isotope Inc., Tokyo, Japan) was used for RIA with a polyclonal LH antibody and World Health Organization second immunoreactive plasma–human menopausal gonadotropin as the standard preparation. A "Spac-S LH-kit"

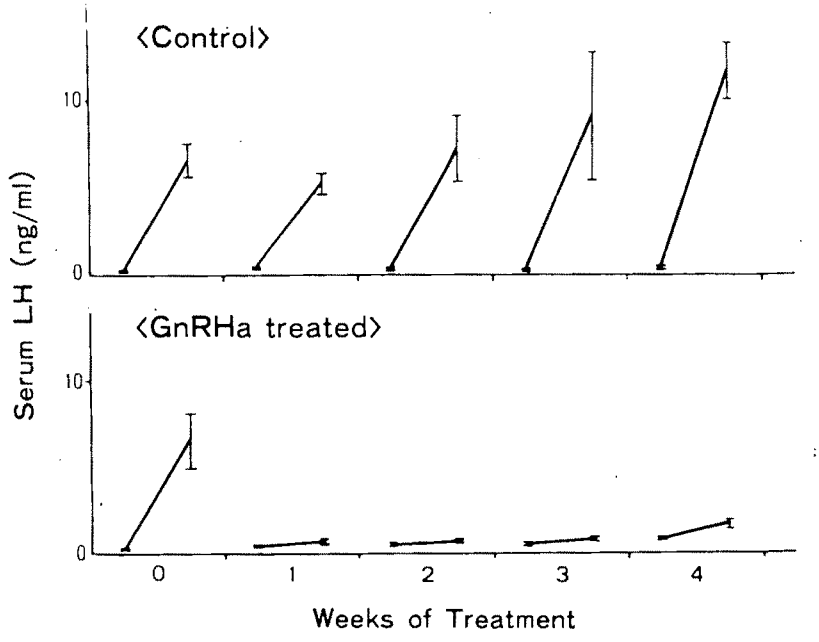


Fig. 7. Serum rat LH before and 30 minutes after native GnRH (1 μg/rat, subcutaneous) administration 0 to 4 weeks after GnRH agonist treatment in intact immature female rats *(upper panel)* and in GnRH agonist–treated immature female rats *(lower panel)* (mean ± SE, $n = 5$).

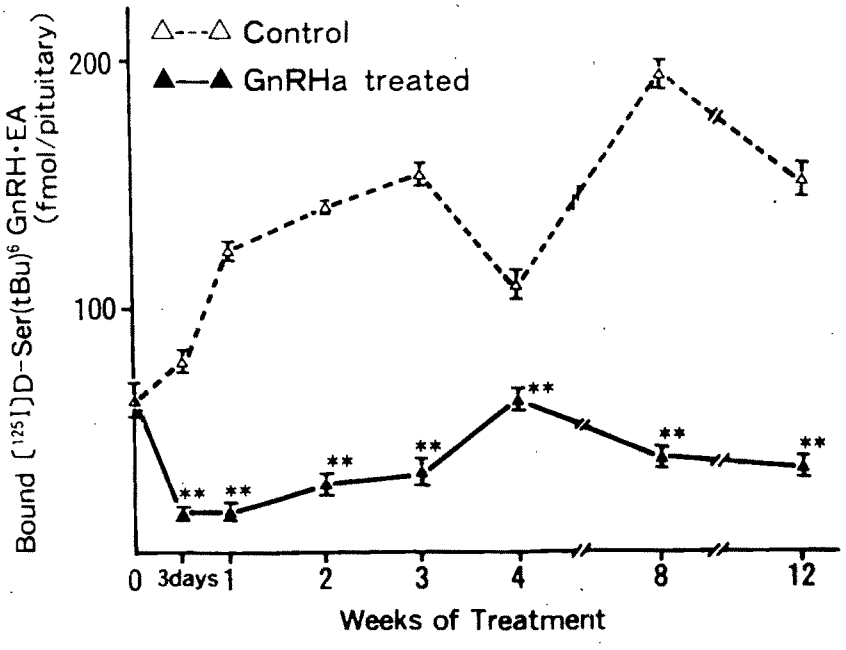


Fig. 8. Effects of GnRH agonist treatment on pituitary GnRH receptor in female rats (mean ± SE, $n = 5$). $^{**}p < 0.01$ compared with controls.

(Daiichi Isotope Inc.) was used for IRMA with monoclonal LH antibodies and World Health Organization first immunoreactive plasma–LH as the standard preparation. For follicle-stimulating hormone (FSH) determinations we used the FSH kit "Daiichi" for RIA with a polyclonal antibody, World Health Organization second immunoreactive plasma–human menopausal gonadotropin and "Spac-s FSH-kit" for IRMA with monoclonal antibodies, and World Health Organization second immunoreactive plasma–human pituitary gonadotropin. Data were subjected to a one-way analysis of variance followed by the Student t test.

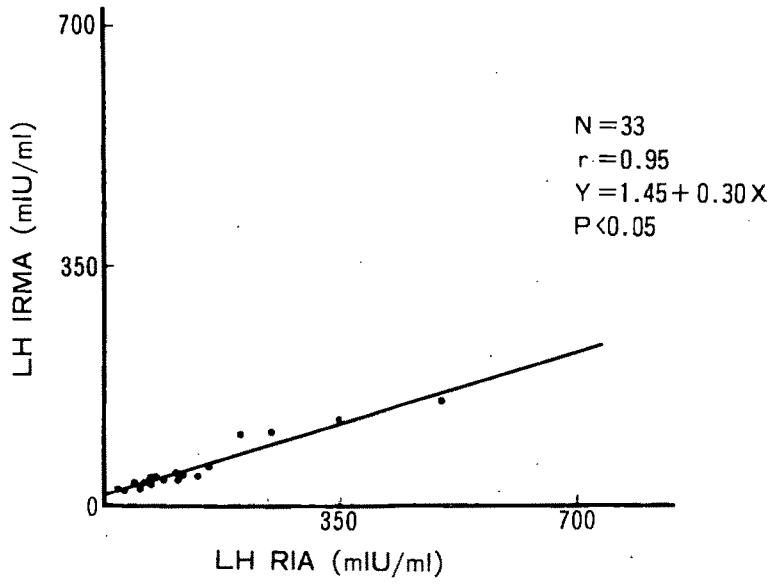


Fig. 9. Correlation between LH values determined by RIA (LH-RIA) and IRMA (LH-IRMA).

Results

Desensitization of pituitary cells in vitro. Pulsatile infusion of 10^{-9} mmol/L GnRH induced pulsatile secretion of rat LH, but continuous infusion of 10^{-8} mmol/L GnRH or 10^{-7} mmol/L GnRH induced a rapid and transit increase of rat LH; thereafter the levels of rat LH decreased to the pretreatment level within 3 to 4 hours (desensitization). The desensitization was also observed when the pulse amplitude of GnRH was more than 10^{-7} mol/L GnRH or when the pulse frequency was more than four times per hour. Even after this continuous infusion of 10^{-8} mol/L or 10^{-7} mol/L GnRH, the pituitary cells responded to 10^{-6} mol/L GnRH, 50 mmol/L K^+, and 3 mmol/L 8-bromo-cyclic adenosine monophosphate (Figs. 1 to 3).

Study in rats in vivo. During the treatment of GnRH agonist (0.94 mg/rat) the ovarian weight was 17.1 ± 1.7 mg per ovary (mean ± SE) on day 0 and 18.3 ± 0.8 mg on day 3. Thereafter the weights decreased significantly to 15.2 ± 0.9 mg (control, 22.9 ± 2.3; $p < 0.05$) on day 7 and 9.2 ± 1.7 mg (33.2 ± 5.8; $p < 0.01$) on day 28 (Fig. 4).

The serum concentration of rat LH increased after hour 1 of treatment ($p < 0.05$) and continued to rise until hour 2 ($p < 0.01$). Thereafter the levels decreased gradually but were higher than those measured in the intact control animals on day 3 ($p < 0.01$). On day 28 the concentrations had returned to the control levels. Serum rat FSH was also the same level as the control (Figs. 5 and 6).

Serum rat LH response to GnRH (1 μg) in GnRH agonist–treated rats decreased significantly ($p < 0.05$) on day 7 compared with the control group, and this blunted response remained unchanged up to week 4 after GnRH agonist administration (Fig. 7).

The pituitary rat LH content decreased gradually after GnRH agonist administration. Three hours after treatment the rat LH content was significantly lower than the basal level; it continued to drop slowly until day 3 and then remained unchanged up to day 28 (Figs. 5 and 6).

GnRH receptors in the pituitary markedly decreased to 17.3 ± 2.6 fmol/mg protein (mean ± SE) compared with the control group (123.2 ± 0.8; $p < 0.01$) on day 7 and remained at low levels (Fig. 8).

The rat LH β-mRNA concentrations in the GnRH agonist–treated group were not significantly different from those in the control group from 1 to 18 hours after GnRH agonist administration, but the mRNA concentrations in the GnRH agonist–treated animals from day 3 to day 28 ($p < 0.05$) were lower than those in the control group ($p < 0.05$) (Figs. 5 and 6).

Clinical study

GnRH agonist (nafarelin) administration to eight patients who had polycystic ovarian disease and seven infertile patients. LH values determined by IRMA (LH-IRMA; Y) and those determined by RIA (LH-RIA; X) correlated significantly ($r = 0.9472$; $p < 0.01$) except for LH values during nafarelin treatment ($Y = 1.45 + 0.3X$) (Fig. 9). Nafarelin decreased LH-IRMA significantly from day 7 to day 21 ($p < 0.01$), but it did not decrease LH-RIA. Therefore the ratio LH-RIA to LH-IRMA (LH-RIA/LH-IRMA) increased significantly from 8.4 ± 1.5 to 17.4 ± 2.3 ($p < 0.05$) at week 3 of nafarelin treatment (Fig. 10).

The mean value of serum estradiol decreased from

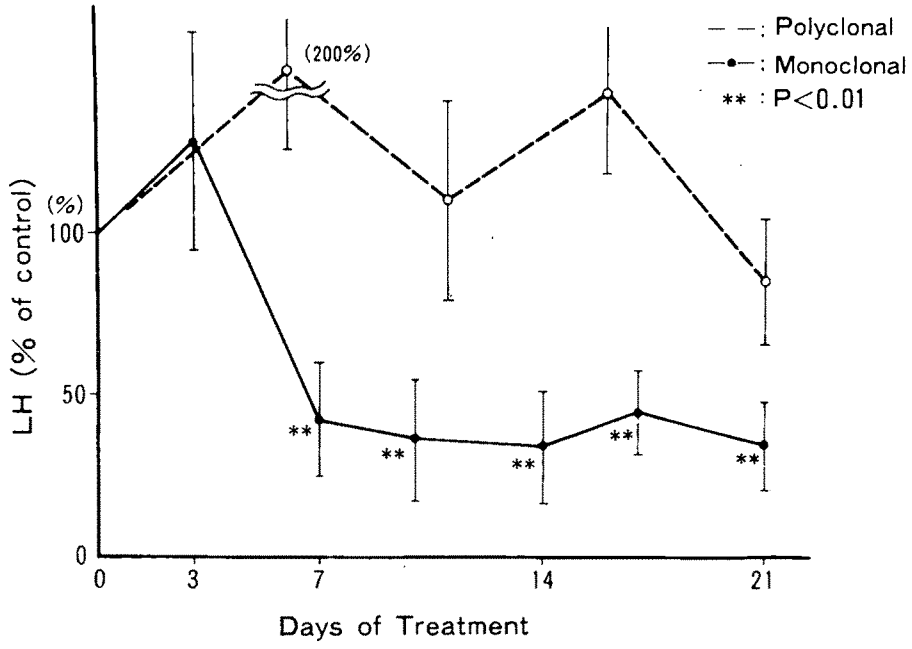


Fig. 10. Changes in LH-RIA, LH-IRMA, and estradiol in blood during treatment with GnRH agonist (nafarelin, 400 μg/day) for 3 weeks (mean ± SE, $n = 15$).

44.1 ± 5.9 pg/ml to 16.3 ± 2.9 pg/ml after treatment; this change was consistent with that of LH-IRMA but not with that of LH-RIA.

GnRH agonist (TAP-144-SR) treatment for patients who had endometriosis. The mean LH-RIA was 20.1 ± 2.6 mIU/ml ($n = 16$) before treatment and did not decrease significantly at week 2 (21.3 ± 1.9), week 4 (16.4 ± 0.6), week 16 (14.3 ± 2.1), or week 20 (15.8 ± 1.8) of treatment. On the other hand, LH-IRMA was 6.5 ± 1.3 mIU/ml before treatment and decreased dramatically from week 2 (3.3 ± 0.5; $p < 0.05$) to week 24 (0.5 ± 0.01; $p < 0.001$) of treatment.

FSH-IRMA also decreased more than FSH-RIA, and the estradiol levels reflected the changes of LH-IRMA and FSH-IRMA more than those of LH-RIA and FSH-RIA (Fig. 11).

Comment

Treatment with GnRH agonist (0.94/mg/rat/28 days) did not decrease serum concentrations of rat LH, but it inhibited rat LH responses to GnRH agonist starting on day 7 and decreased ovarian weights significantly from day 7 to day 28. Moreover, we have observed that these dosages of GnRH agonist delayed vaginal opening in immature female rats (unpublished data). These findings indicate that the dosage of GnRH agonist used in this study was large enough to induce pituitary desensitization within 7 days.

After treatment with GnRH agonist, the GnRH agonist binding sites decreased significantly compared with those of controls. In the in vitro study desensitized gonadotropes responded to potassium, a high dose of GnRH, and a cyclic adenosine monophosphate derivative, 8-bromo-cyclic adenosine monophosphate, which could clearly induce rat LH secretion in nontreated pituitary cells.[1] These results suggest that the content of pituitary rat LH was not exhausted and that desensitization was mainly caused by an alteration at the receptor level.

The mRNA of the rat LH β-subunit appears to be an important rate-limiting factor in the synthesis of mature gonadotropin; therefore the changes in rat LH β-mRNA were examined during the down-regulatory phase of GnRH agonist treatment. This study showed that reduced availability of rat LH β-mRNA was an important factor that contributed to the depletion of rat LH content in the pituitary from GnRH-desensitized rats, although rat LH β gene expression was not involved in the marked reduction in pituitary content during the early stage of GnRH agonist treatment.

In the clinical study different groups were given different types of agonists; therefore comparison between patient groups is not appropriate. The differences between continuous and subcutaneous administration versus intermittent intranasal administration are important, because continuous administration of GnRH agonist has been shown to result in greater inhibition of LH secretion than intermittent administration.[5] Moreover, the dosage of GnRH agonist (TAP-144-SR

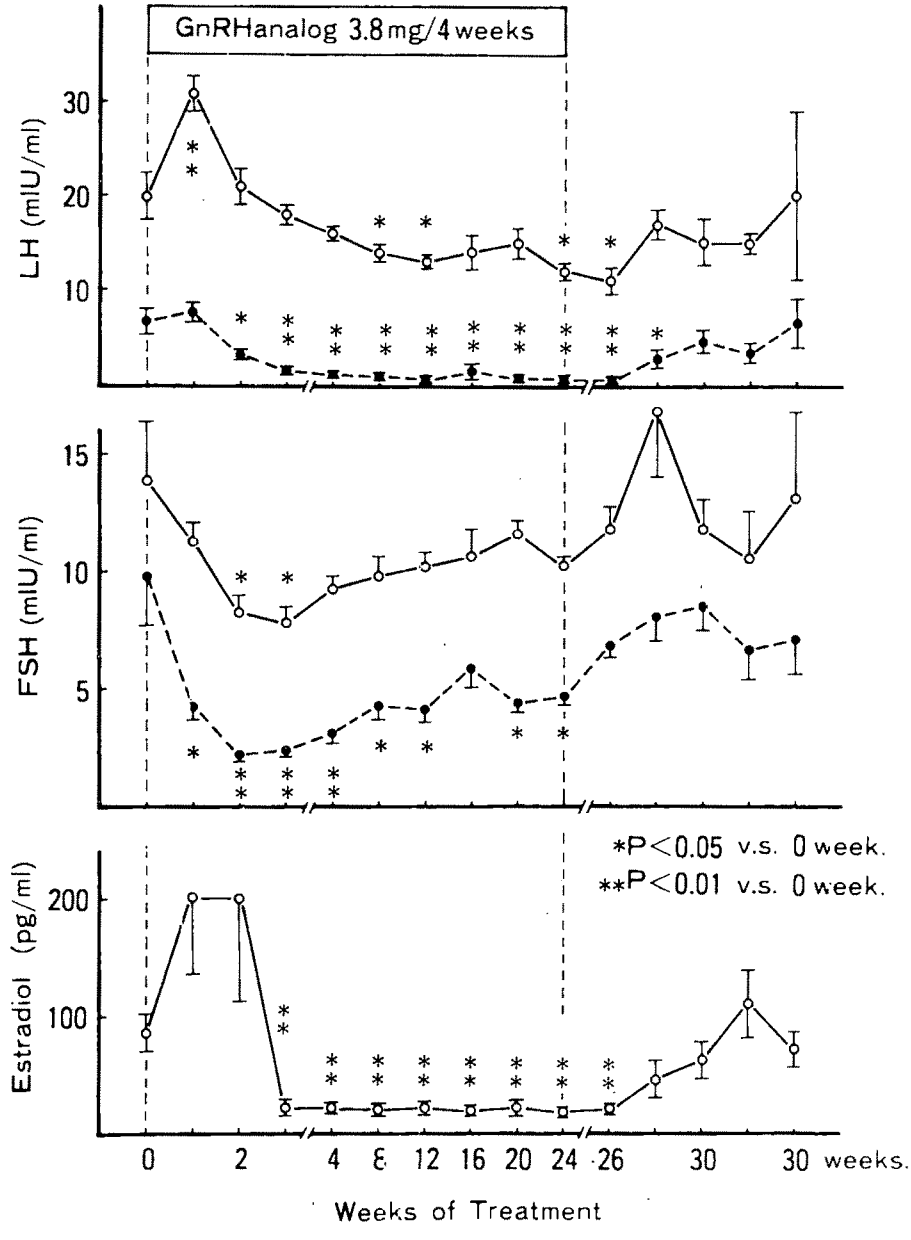


Fig. 11. Changes in LH-RIA, LH-IRMA, FSH-RIA, FSH-IRMA, and estradiol in blood during treatment with GnRH agonist (TAP-144-SR 3.8 mg/4 wk, subcutaneous) for 24 weeks (mean ± SE, $n = 16$).

3.8 mg/4 wk, subcutaneous) was larger than that of GnRH agonist (nafarelin 400 μg/day, intranasal), because the absorption rate of intranasal administration is about 1% to 10%.[6] Therefore TAP-144-SR suppressed LH-RIA and LH-IRMA more than nafarelin.

In this study a disparity between LH-RIA and LH-IRMA in the process of gonadotrope desensitization to GnRH was demonstrated; that is, the levels of LH-RIA did not change markedly, but LH-IRMA decreased dramatically during treatment with GnRH agonist. The changes in LH-IRMA were consistent with those of serum estradiol. The mechanisms involved in this disparity after GnRH agonist treatment are uncertain, but increases of immunoreactive molecules such as α-subunit and LH fragments might be related. These findings are consistent with the data presented in previous reports, that is, that unchanged immunoreactivity of serum LH after GnRH agonist treatment is partially caused by the cross-reactivity of free α in the LH-RIA[7] and that common α-subunit gene expression is sustained in the presence of a pharmacologic concentration of GnRH agonist.[8]

In the desensitized pituitary FSH-IRMA was also significantly suppressed from week 1 to week 24 and correlated well with estradiol levels; however, FSH-RIA suppression was significant only at weeks 2 and 3. It

was reported that rat FSH β-mRNA levels were markedly reduced when gonadotropes were desensitized by GnRH agonist and that the effects of GnRH agonist on FSH β-nRNA levels were paralleled by its effects on LH β-mRNA levels.[9] These data also suggested increases of immunoreactive molecules such as α-subunit and FSH fragments.

These data suggested involvement of a postreceptor mechanism or non-GnRH receptor–mediated mechanisms, in addition to a receptor mechanism in desensitization, although chemical characterization should be investigated to elucidate the molecular heterogeneity observed during GnRH agonist treatment.

We gratefully acknowledge receipt of the rat LH kits from the National Hormone and Pituitary Program, NIDDK. GnRH analogs TAP-144-SR, buserelin, and nafarelin were kindly provided by Takeda Chemical Industries, Ltd., Hoechst Japan Co., Ltd., and Nippon Syntex KK.

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LETTERS TO THE EDITORS

Myocardial necrosis in a newborn after long-term maternal subcutaneous terbutaline for suppression of premature labor

To the Editors: We read with interest the report by Fletcher et al. (Fletcher SE, Fyfe DA, Case CL, Wiles HB, Upshur JK, Newman RB. Myocardial necrosis in a newborn after long-term maternal subcutaneous terbutaline infusion for suppression of preterm labor. AM J OBSTET GYNECOL 1991;165:1401-4) detailing a case of neonatal myocardial necrosis possibly caused by prolonged subcutaneous terbutaline tocolysis. Unfortunately, the case as presented lacks a number of cogent facts that may have had an impact on the occurrence of fetal adverse events. For instance, during the subcutaneous therapy how rapid was the fetal heart rate (as a possible indication of fetal drug level)? How frequent were uterine contractions during the reported 12-week time period (recognizing the importance of repetitive obstruction of the uterine circulation on the fetus, compression of the umbilical cord, or both)? What was the status of the patient's diabetes during this interval (realizing the effect hyperglycemia, ketosis, or both, may have on the fetal myocardium)? And finally, what transpired at delivery (e.g., length of labor, route of delivery, Apgar scores, cord pH)? We would find it extremely difficult to evaluate adequately any event in the neonatal course with the paucity of antenatal and intrapartum data given in the article.

However, even more alarming than the lack of information was the astoundingly high dosage of terbutaline used both initially and during the last 2 weeks of gestation. The usual initial dose of terbutaline given subcutaneously via infusion pump is a basal rate of 0.050 mg per hour.[1] Even when it is taken into consideration that the bioavailability of terbutaline given subcutaneously is in excess of 90% (compared with oral drug), the 0.50 mg per hour reported by the authors is literally an overdosage by a factor of 10. Added to this are bolus doses of 0.3 mg per hour, resulting in a total dose of 0.80 mg per hour. This yields a total daily dose of 19.2 mg per day for the last 2 weeks of pregnancy. This approaches the oral dosages used by some authors; these oral dosages can be clinically ineffective after short intervals (not to mention the differences in bioavailability).[2] Because it is well known that any adverse cardiac effects of β-sympathomimetic drugs are in part related to total dosage, it would be no surprise if the mother or the infant were to have some ill effects of this clearly excessive level of terbutaline.

In conclusion, we reiterate that the report by Fletcher et al. is, first, difficult to analyze conclusively given the lack of appropriate obstetric information. Second, because the usual total daily dose of subcutaneous terbutaline for tocolysis (including both basal rate and bolus dosages) is on the order of 3.0 to 5.0 mg per day, it is not surprising that a triple or quadruple overdose would have resulted in an adverse event for either mother or baby. This result should not negatively influence obstetricians planning to use subcutaneous terbutaline in appropriate doses for long-term tocolysis.

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Reply

To the Editors: We thank Bey et al. for their comments. They have raised some interesting points regarding parts of the obstetric history that were omitted in our manuscript because of editorial limitations. The patient's antepartum records indicated that she began receiving subcutaneous terbutaline pump therapy at approximately 24 weeks' gestation and continued through 36 weeks' gestation. The subcutaneous terbutaline infusion pump was discontinued at approximately 36 weeks because of significant maternal side effects, including jitteriness and tachycardia. Fetal tachycardia was not identified during the antepartum period, with the fetal heart rate recorded as being within normal limits at each antepartum visit. Unfortunately the data on the frequency of uterine contractions recorded by the home uterine activity monitoring device during the 12 weeks of subcutaneous therapy are not available to us. However, the antepartum record indicates that the patient's uterine contractions exceeded protocol limits on a daily basis. Antepartum uterine activity monitoring performed in the obstetrician's office revealed "strings of contractions."

The patient also had diet-controlled gestational diabetes. The results of a glucola screen were 145 mg/dl. This test was followed by a standard oral 3-hour glucose tolerance test that revealed abnormal values at 2 and 3 hours. The patient given a 2000-calorie American Dietetic Association diet and follow-up fasting; 2-hour postprandial blood glucose levels were <100 mg/dl. The patient was admitted at 37 weeks' gestation in latent phase labor with the cervix dilated to 3 cm. After several hours without cervical change, oxytocin augmentation was begun. Labor progressed over the ensuing 8 hours resulting in delivery of a normal spontaneous vaginal delivery of a 6 lb. 11 oz. infant with Apgar scores of 9 and 9 at 5 and 10 minutes. Neither umbilical artery nor umbilical vein cord gases were obtained. The fetal heart rate monitoring was reported as being normal throughout labor, and the only analgesia used was a combination of propiomazine hydro-

chloride (Largon) and nalbuphine hydrochloride (Nubane) during the active phase of labor.

We agree with Bey et al. that the dosages of terbutaline used both initially and during the last 2 weeks of gestation, when the patient complained of increased heart rate, palpitations, tremulousness, and anxiety, were in excess of those dosages commonly used with subcutaneous terbutaline pump therapy. The concerns of Bey et al. that the ill effects suffered by this infant may be attributable to this excessive level of terbutaline is obviously well taken.

The purpose of our article was to report the occurrence of myocardial necrosis that we *speculate* was caused by the subcutaneous terbutaline tocolytic infusion. The obstetric literature has reported success with this type of therapy, and the importance of avoiding premature delivery of infants is obvious. As with any medication or intervention, the risk/benefit ratio should always be considered when prescribing. We hope that this case report will stimulate other obstetricians and pediatricians to report similar occurrences and to initiate scientific investigations as to the effect of continuous subcutaneous terbutaline infusion on fetal and neonatal cardiovascular performance.

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Fetal choroid plexus cysts and trisomy 18

To the Editors: In their article Platt et al. (Platt LD, Carlson DE, Medearis AL, Walla CA. Fetal choroid plexus cysts in the second trimester of pregnancy: a cause for concern. Am J Obstet Gynecol 1991;164:1652-6) argue that it is reasonable to offer amniocentesis to all patients who have ultrasonographically identified choroid plexus cysts in the second trimester. This view is supported by the available prospective literature. Nine prospective studies (including that of Platt et al.) provide data sufficient to calculate predictive values.[1-8] A total of 35,668 patients have been screened in the second trimester, with 324 fetuses having choroid plexus cysts (prevalence 0.9%). Excluding the other chromosomal aneuploidies identified, 11 (3.4%) of the 324 fetuses with choroid plexus cysts had trisomy 18. If 70% of these fetuses had ultrasonographically identified anomalies that characterized them as being at increased risk for trisomy 18,[9] then 3 (0.9%) of 324 would have choroid plexus cysts as the only ultrasonographically detected abnormality (predictive value 1 in 108). This number lies within current thresholds for amniocentesis and also corresponds closely to theoretical projections based on a sensitivity of 30%, a specificity of 99.1%, and a prevalence of 1 in 1000 for second-trimester trisomy 18.[10] The theoretic predictive value of 3.2% is independent of associated anomalies.

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Reply

To the Editors: Bernstein has nicely reviewed the prospective literature with regard to the identification of a choroid plexus cyst as an indication for amniocentesis. I concur that the main purpose of our article was to emphasize that in spite of sophisticated ultrasonography, not all malformations will be detected. Although Bernstein's review does not include this premise, the data he presents further emphasize the correlation of aneuploidy with choroid plexus cysts even in the absence of identifiable malformations. I believe that his comments and our article continue to support offering amniocentesis to these patients in spite of the apparent risk of this procedure. I thank Bernstein for his timely comments.

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Laser therapy for intraepithelial cancer of the vagina

To the Editors: We were most interested in the article by Hoffman et al. (Hoffman MS, Roberts WS, LaPolla JP, Fiorica JV, Cavanagh D. Laser vaporization of grade 3 vaginal intraepithelial neoplasia. Am J Obstet Gynecol 1991;165:1342-4) because we recently published an article on the same subject.[1]

The conclusions of Hoffman et al. are the complete opposite of ours. We do agree with Hoffman et al. that careful evaluation and an accurate appraisal of the ex-

tent and severity of the disease should be made before laser therapy and that every mode available for evaluation and appraisal should be used. Multiple selected biopsies are most important to rule out the possibility of underlying invasive disease.

However, we believe that once invasive disease has been ruled out a laser ablation of the affected area and its immediate surrounding tissue is the best way to eliminate preinvasive disease and give the patient the best result with the least degree of morbidity and complications and with improved subsequent sexual function. Some authors[1] who have dealt with this problem cite the difficulty of the technical ability to eradicate adequately those pits and deep scars that may occur in the vault of the vagina and in the corner recesses. We believe our technique allows us to apply the laser therapy accurately and adequately to accomplish the result. The use of appropriately placed wire sutures allows better visualization and accessibility of the laser beam to the tissue. In addition, the use of submucosal infiltration allowed us to accomplish our published results. Since the publication of our article our continued successful results have convinced us of the appropriateness of the procedure with the guidelines suggested. Furthermore, we are sorry that Hoffman et al. did not find it convenient to include our article in their list of references.

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REFERENCE

1. Sherman AI. Laser therapy for vaginal intraepithelial neoplasia after hysterectomy. J Reprod Med 1990;35:941-4.

Reply

To the Editors: I indeed overlooked Sherman's article, and I apologize to him for this. I have now reviewed his article and find that it contains a large series of patients who were successfully treated by laser vaporization.[1] The successful treatment in his study was attributed to patient selection, infiltration of fluid into the submucosa, and the use of wire sutures for exposing scarred and recessed areas. We have also used submucosal infiltration in some patients and have found it helpful for hemostasis, but we have not used it in the manner described by Sherman. We and others have also used skin hooks in a manner probably similar to the wire sutures to gain exposure to recessed areas. We find the techniques described by Sherman to be interesting and will certainly consider using them in the future. Our findings and conclusions on the basis of our experience with grade 3 vaginal intraepithelial neoplasia are quite different from those of Sherman's, and there may be reasons for these differences besides technique. Although all of our patients had grade 3 intraepithelial neoplasia, it is unclear whether this was the case in Sherman's paper. Our study was much smaller than Sherman's and it would not be appropriate to make a more in-depth comparison between the two studies. We, however, are particularly concerned about the use of laser vaporization for grade 3 vaginal intraepithelial neoplasia diagnosed at or near a vaginal cuff scar, particularly when there is marked distortion around this area or when the previous hysterectomy had been done for squamous cell neoplasia. In our limited experience with such patients we have seen a high rate both of recurrence and of occult invasive carcinoma.[1-3]

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Initiation of antepartum testing in the hypertensive gravid woman

To the Editors: We read with interest the article by Pircon et al. (Pircon RA, Lagrew DC, Towers CV, Dorchester WL, Gocke SE, Freeman RK. Antepartum testing in the hypertensive patient: when to begin. Am J Obstet Gynecol 1991;164:1563-70). We are concerned that their conclusions are not substantiated by the data as presented. The stated purpose of their investigation was to determine the gestational age at which antepartum testing should begin in various subgroups of hypertensive gravida women. However, the data do not tell the reader how many or with what frequency antepartum tests were done at various gestational ages.

We are concerned that the study analyzed the data as the percentage of abnormal antepartum tests *per number of patients*. To determine the sensitivity and specificity of any test, the results must be analyzed as the percentage of abnormal tests *per number of tests performed* at a given gestational age. Only in this way can meaningful conclusions be drawn regarding the differential use of these tests in various subgroups of patients with hypertension. For example, if diabetic and nondiabetic patients with hypertension are equally at risk for abnormal test results but the patients with diabetes are tested earlier in pregnancy and more often, it is to be expected that the patients with diabetes would have a higher percentage of abnormal test results *per patient*. However, the patients with diabetes would have the same percentage of abnormal test results *per number of tests performed*.

In addition, we were unable to delineate the test results by group and by subsequent management. Are the 53 patients with positive contraction stress tests included in the 119 with "abnormal test results"? Were the abnormal test results separated into the categories of abnormal contraction stress test, abnormal nonstress

test, and abnormal biophysical profile? The data also fail to indicate how often and under what circumstances an abnormal test result was ignored and the pregnancy allowed to continue for an extended period.

The statement of Pircon et al. that antepartum testing in diabetic patients with hypertension should be started at viability is apparently based on the two late second-trimester stillbirths that occurred during the study period. Pircon et al. acknowledge that both fetuses had true knots in the umbilical cords; these knots could have had a role in their deaths. Are there data demonstrating that diabetic patients with hypertension are at increased risk of umbilical cord knotting resulting in stillbirths?

The question of when to begin fetal testing in patients with hypertension is extremely important. A retrospective analysis could be quite informative. We believe that the data presented by Pircon et al. should be reanalyzed, taking into account the number of tests performed at any given gestational age, with a more complete data presentation. Then justifiable conclusions could be drawn as to when antepartum testing should be initiated in the patient with hypertension.

Avick G. Mitra, MD, and William N.P. Herbert, MD
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Reply

To the Editors: We thank Mitra and Herbert for their interest in our study of antepartum testing in patients with hypertension. The answers to their questions can be found in the text of our original publication; however, we are happy to review the results again. Our objectives were to evaluate when fetal compromise occurs in this patient population and to make recommendations as to when to initiate antepartum testing.

Contrary to the suggestion of Mitra and Herbert, the data were not analyzed as a percentage of abnormal antepartum tests per number of patients. We were not interested in the age-old issue of sensitivity or specificity of a testing method in patients with hypertension; rather, we were interested in when compromise occurs. We defined compromise by several parameters, one of which was a positive contraction stress test. Test results were recorded prospectively and were retrospectively queried by first positive test, not by the number of positive tests the patients had. In evaluating the data in this fashion we found that hypertensive patients with concomitant diagnoses of diabetes demonstrated compromise at an earlier gestational age when compared with hypertensive patients without diabetes.

Subsequent management was not the topic of study. When patient management is modified because of an antepartum test, there is evidence of fetal compromise. To reiterate, we were interested in when this occurred. We all agree that follow-up of an abnormal test can be managed numerous ways. Although it seems that Mitra and Herbert have expressed an interest in our management protocol, that protocol would not affect our results or our conclusions in this study. Consequently we do not believe it is appropriate to review that topic here.

We recommended that testing in the diabetic patient with hypertension start at viability. As listed in our original publication, this was based on two observations. First, there were two patients with late second-trimester stillbirths and concomitant diagnoses of a true knot in the umbilical cord. It is difficult to attribute the losses solely to the knots in the cords because this is a common finding at the deliveries of normal, viable term infants. We did not suggest that there is an increased risk of cord knotting in diabetic patients with hypertension. Second, a clinician would prefer to initiate testing in a patient before fetal compromise. The earliest gestational age that intervention was necessary in the diabetic patient with hypertension was 28 weeks. Therefore testing would need to start at 26 to 27 weeks or near the time of viability.

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Legal thoughts on malpractice claims: Causes and prevention

To the Editors: As a professor of consumer law married to a physician, I read with great interest Ward's article (Ward CJ. Analysis of 500 obstetric and gynecologic malpractice claims: causes and prevention 1991; 165:298-306). I believe the article would have been improved and Kirkley's comments muted by a change in the classification method. Ward divides the cases into "defensible" and "indefensible," and Kirkley has a number of problems with this categorization. So do I, but for a different reason. There is simply no division in the law which corresponds to the defensible-indefensible dichotomy. The law divides cases into three categories, not two. In the first the plaintiff has no case and the defendant wins on summary judgment. In the second the defendant has no case and the plaintiff wins on summary judgment. In the third the issue is presented to the jury for determination of the disputed facts. Given Ward's description of his method it would appear that most, though not all, of his indefensible cases fall into the second category, where the plaintiff might win on summary judgment. The defensible cases would be in the other two categories. However, even if a case can be made for a physician's actions, it does not mean that the physician will win.

Ward's article may give physicians the unwarranted belief that if they lose a "defensible" case, the system has somehow failed them presumably because of perjured expert testimony, idiot jurors, and corrupt lawyers. This is simply not the law, just as it is not the law that a bad outcome means that there was malpractice. The legal system recognizes that there are often factual disputes on which reasonable people can differ and

assigns the resolution of such disputes to juries. The burden of proof is only the preponderance of the evidence that the physician violated the appropriate standard of care. In a standard of preponderance of the evidence we accept that there will be a certain number of "incorrect" verdicts and that verdicts do not establish truth but only solve the operative question of who wins the case. This type of error is inherent in the legal system. Including this understanding in the method used in the research would make the conclusions of much greater practical use.

I do not mean this comment to imply criticism of the obvious effort and skill Ward brought to this difficult issue and the real value of his advice to physicians (I assume the statement that human papillomavirus is "not" a likely cause of cervical cancer is a typographical error). However, I have a few replies to the comments of the other discussants.

Kirkley confuses the "standard of care" required by the law with the practice patterns of the "20,000 to 30,000 members of the American College of Obstetricians and Gynecologists who actually establish the standard of care in their localities." The standard of care is not how the typical physician practices any more than it is how the typical driver drives. The typical driver is negligent some of the time. Good drivers and good physicians sometimes make mistakes, with terrible consequences. The standard of care is an abstract ideal, and it is possible for an entire profession or industry to fall below a standard of care at least some of the time.

I am surprised that Kirkley's admonitions against changing medical records are seemingly based on the probability of being caught rather than the fact that changing records is a disgraceful, unethical act by anyone who claims to be a physician. I also question his statement that you "never tell a patient that you did wrong." Physicians owe patients a fiduciary duty to disclose errors. Failure to comply with this duty is itself actionable, and more practically may extend the statute of limitations under a theory of fraudulent concealment.

As a professor I was appalled that Blanchard would try to use a department chair to "take a stand" against an expert testifying in litigation. Virtually all states have civil or criminal penalties for those who tamper with witnesses in civil litigation, and any decent university respects the academic freedom of its professors.

Ward's article is a useful addition to the medical-legal literature. I only add that the real goal is not to avoid liability, but to avoid the injury that leads to the claim of liability.

Vincent Brannigan
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Reply

To the Editors: I appreciate Brannigan's comments and attention to my manuscript. His comment concerning human papillomavirus was correct. The "not" was a typographic error. The sentence from my article should have read "Human papillomavirus is *now* considered to be a likely cause of cervical cancer, even though Koch's postulates have not been fulfilled."

I recognize the insightful comments regarding definitions of "indefensible" and "defensible." In this study all the claims reviewed were open claims and not settled cases. Judgments were made without the benefit of the opinion of a jury; therefore the third category referred to by Brannigan could not have been used.

In reviewing a claim, if the medical evidence or lack of same revealed a breach in the standard of care resulting in damage, then the claim was deemed indefensible and settlement was advised. However, if malpractice did not exist, I classified the case as defensible. In these latter claims I recommend that the case be defended, even if a defendant's summary judgment was not obtained.

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Laparoscopic hysterectomy: Reinventing the wheel?

To the Editors: Laparoscopic surgery is back on the scene. Ten years ago it might have been predicted that laparoscopy would be a dying procedure replaced by more advanced assisted reproductive technologies. However, the development of conventional[1] and laser[2, 3] laparoscopic accessories and the incorporation of video imaging technologies[4] into the armamentarium of the endoscopic operating theater has made it possible to perform more radical procedures safely and to establish training programs for advanced laparoscopy.

On the other hand, aggressive marketing of medical treatments coupled with public knowledge fed by eager journalism may be creating a misinterpretation of "new" applications such as "laparoscopic hysterectomy." At a recent international scientific event one of the keynote speakers stated " . . . none of my patients accept any longer the idea of conventional hysterectomy." These types of comments subsequently become headlines in women's magazines, proclaiming "New way of removing your womb."

The situation is reminiscent of Hans Christian Andersen's famous story, "The Emperor's New Clothes." Is this procedure new? Or is it in fact a laparoscopy-assisted vaginal hysterectomy or sometimes a laparoscopy-monitored vaginal hysterectomy? An experienced vaginal surgeon can perform a vaginal hysterectomy (with or without adnexectomy[5]) in <25 minutes.[6] Sir Norman Jeffcoate stated, "vaginal hysterectomy has an important place, not as a part of an operation for prolapse, but as the best means of removing the uterus when prolapse is not present."[7] Although there are indications in which laparoscopic assistance for adhesiolysis, confirming diagnosis, or minimizing complications are beneficial, it is doubtful that potential candidates for surgery who prefer the "modern approach" are even aware of proper patient selec-

tion. In a recent presentation of supracervical (real) laparoscopic hysterectomy[8] the question of its place and potential hazards were critically discussed.

Having been involved with the development of endoscopic accessories and techniques in the last 13 years, I truly believe that future surgery is connected to endoscopes. The sooner a gynecologist becomes familiar with the endoscopic equipment, different hand-eye coordination with video monitors, and proper patient selection the better. However, as one of Joel Cohen's past residents, I believe that the following message should be delivered: Let's not (1) forget the classic approach or (2) reinvent the wheel or even replace it with "square wheels." Let's be honest with each other and recognize what is really new. Present technology offers enough room for new ideas.

Yona Tadir, MD

Department of Obstetrics and Gynecology and Beckman Laser Institute and Medical Clinic, 1002 Health Sciences Road East, Irvine, CA 92715

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ITEMS

Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $60.00 U.S. per insertion and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to Kay G. Goehler, Senior Manuscript Editor, Journal Editing, Mosby–Year Book, Inc., 11830 Westline Industrial Drive, St. Louis, MO 63146-3318.

The Harvard Medical School, Department of Continuing Education, and The Massachusetts General Hospital, Department of Gynecology, are presenting a postgraduate course in: Vaginal Surgery, directed by David H. Nichols, MD, on October 9, 10, 11, 1992, at The Ritz-Carlton Hotel, Boston, Massachusetts. For information contact: Harvard Medical School, Department of Continuing Education. Tel.: (617) 432-1525.

Tenth BIRTH Conference, Innovations in Perinatal Care: Assessing Benefits and Risks, October 31–November 1, 1992, Boston Park Plaza Hotel, Boston, Massachusetts. For information please contact: Diony Young, Editor, *Birth,* 43 Oak St., Geneseo, NY 14454. Tel.: (716) 243-0087.

Courses sponsored by University of California, Irvine, and Long Beach Memorial Medical Center. For information contact the Center for Health Education. Tel.: (310) 595-3811.

July 29–August 1, 1992, 10th Controversies OB/GYN, Mauna Kea, Hawaii
November 16-21, 1992, 15th Annual OB/GYN Review, Costa Mesa, California
January 13-17, 1993, 8th Annual Perinatal Symposium, Beaver Creek, Oregon
February 27–March 6, 1993, 14th Annual Current Topics in OB, Aspen, Colorado
March 25-27, 1993, Gynecologic Surgery, Rancho Las Palmas, California

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Bound volumes of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY are available to subscribers (only) for the 1992 issues from the Publisher, at a cost of $68.00 for domestic, $95.76 for Canada, and $91.00 for international for Vol. 166 (January-June) and Vol. 167 (July-December). Shipping charges are included. Each bound volume contains a subject and author index and all advertising is removed. Copies are shipped within 60 days after publication of the last issue in the volume. The binding is durable buckram with the JOURNAL name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact Mosby–Year Book, Inc., Subscription Services, 11830 Westline Industrial Drive, St. Louis, MO 63146-3318, USA; phone (800) 325-4177, ext. 4351, or (314) 453-4351.

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WOMEN & INFANTS HOSPITAL OF RHODE ISLAND
Department of Obstetrics and Gynecology
Specialist in Maternal-Fetal Medicine and Genetics

Women & Infants Hospital of Rhode Island, is seeking a physician to serve as a specialist in Maternal-Fetal Medicine and Genetics. The Hospital cares for 9500 births per year and houses a 65 bed Level III neonatal unit. The Division is composed of five MFM faculty, an internist, two MFM fellows and two Masters level genetic counsellors. Division staff actively collaborate with a biochemist and a cytogeneticist at WIH, both of whom are active, funded researchers focused on prenatal diagnosis. The Division is housed in newly constructed office space in the hospital and has a new 6000 square foot Prenatal Diagnosis Center providing genetic counselling, sonography, amniocentesis, CVS and PUBS. The person assuming this position will function as Director of Prenatal Diagnostic Services and will also be expected to share clinical responsibilities for MFM patients. Candidates must be certified in or be active candidates for Medical (or Clinical) Genetics (American Board of Medical Genetics) and Obstetrics and Gynecology (American Board of Obstetrics and Gynecology) at the time of taking the position. Completion of an approved fellowship in Maternal-Fetal medicine is highly desirable. The candidate should be actively engaged in a defined area of research, have a proven interest in medical education, and qualify for a full-time appointment at the rank of Assistant or Associate Professor at Brown University. Women & Infants Hospital and Brown University are equal employment opportunity institutions.

Interested parties should send their curriculum vitae and the names of three references to Marshall W. Carpenter, Director of Maternal-Fetal Medicine, Women & Infants Hospital of Rhode Island, 101 Dudley Street, Providence, Rhode Island 02905-2401 by September 1, 1992. Screening will begin on that date and continue until a candidate is selected or the search is closed.

FACULTY POSITION IN OB/GYN MATERNAL-FETAL MEDICINE

Wright State University School of Medicine, Department of Obstetrics and Gynecology, invites applications for a full-time faculty position in maternal-fetal medicine at the Assistant/Associate Professor level.

Primary faculty duties will consist of medical student and resident teaching and supervision. Other duties will include full participation in clinical care activities of the night and week-end coverage of Labor and Delivery. The candidate must be licensable to practice medicine in Ohio. Other qualifications include board certification in obstetrics and gynecology and completion of a fellowship and board certification or active candidacy in Maternal-Fetal Medicine. Academic rank and salary dependent upon qualifications. Women and minorities are encouraged to apply. Please send curriculum vitae and the names and addresses of three references to:

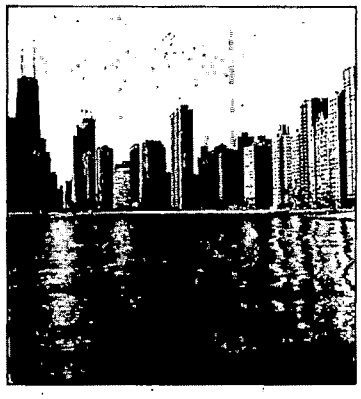
Dennis D. Barber, M.D.
Chair and Program Director
Department of Obstetrics and Gynecology
Wright State University School of Medicine
One Wyoming Street
Dayton, Ohio 45409

All applications received by August 15, 1992, will be considered; if the position is not filled therefrom, applications will be considered as received until the position is filled. Wright State University is an Affirmative Action and Equal Opportunity employer.

Specialty Review in Obstetrics and Gynecology: Practical Aspects

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The University of Louisville Department of OB/GYN is actively recruiting faculty (rank open) to its MFM Division. An attractive balance of teaching, research and clinical activity with a very competitive income is offered. The U of L is an Equal Employment Opportunity institution. Interested individuals should send curriculum vitae to me or contact me at (502) 588-1913.

J. A. Spinnato, M.D.
Director, Division of Maternal-Fetal Medicine
Dept. of OB/GYN,
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FACULTY POSITION REPRODUCTIVE ENDOCRINOLOGY

The Department of Obstetrics, Gynecology & Reproductive Sciences, University of Manitoba and Health Sciences Centre is seeking a Reproductive Endocrinologist for a contingent geographical full time position to assist in providing a full range of tertiary clinical service, resident and medical student teaching, and to participate in research activities. Candidates must have Senior specialty qualifications in Obstetrics and Gynecology in the country of current practice, 2 years subspecialty training or related experience in Reproductive Endocrinology, and be eligible for registration with the College of Physicians and Surgeons of Manitoba. Certification in Obstetrics and Gynecology by the Royal College of Physicians and Surgeons of Canada is preferred. Experience in I.V.F., Endoscopic Surgery, clinical teaching and research desirable. Salary and academic rank will be commensurate with experience and qualifications.

The University of Manitoba encourages applications from qualified women and men, including members of visible minorities, aboriginal people, and persons with disabilities. The University offers a smoke free environment, save for specially designated areas. Priority consideration will be given to Canadian citizens and permanent residents.

Interested candidates should apply in writing by AUGUST 15, 1992, enclosing a curriculum vitae, to: JEREMY KREDENTSER, M.D. UNIVERSITY OF MANITOBA, DEPARTMENT OF OBSTETRICS & GYNECOLOGY, 59 EMILY STREET, WINNIPEG, MANITOBA, R3E 1Y9, CANADA.

Chief Of Obstetrics And Gynecology

The Stamford Hospital, a 305 bed teaching hospital located in southern Connecticut, is seeking a Chief of the OB/GYN service. The Stamford Hospital is a teaching affiliate of the New York Medical College. The primary focus will be the management of the 8 slot residency program and its integration with 3 other teaching programs.

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The Stamford Hospital

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Royal Hospital for Women

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is seeking applicants for two (2) full-time faculty positions for a new women's health center and academic program at its Milwaukee Clinical Campus.

Sinai Samaritan Medical Center has recently consolidated its obstetrical and gynecological services in a redesigned facility in downtown Milwaukee. The department supports a residency program, the largest perinatal service in Wisconsin (5500 deliveries), consultative ultrasound, genetic and infectious disease programs, and reproductive/endocrine services (including an IVF program). The department is affiliated with Planned Parenthood of Wisconsin and St. Luke's Medical Center in Milwaukee.

Qualifications include a board candidacy or certification in obstetrics and gynecology and a commitment to teaching residents, medical students, and clinical research. Qualifications for the two positions for GENERAL OB/GYN can take into consideration special individual interests. Special expertise of interest for the department are:

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Chester B. Martin, M.D.
Chairman
Department of Obstetrics and Gynecology
University of Wisconsin Medical Schl.
600 Highland Avenue, H4/654 CSC
Madison, WI 53792

Fredrik F. Broekhuizen, M.D.
Chairman and Chief
Milwaukee Campus
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Send inquiry and curriculum vitae to: Richard L. Sweet, M.D., Professor and Chairman, Department of Obstetrics, Gynecology and Reproductive Sciences, University of Pittsburgh, School of Medicine, Magee-Womens Hospital, 300 Halket Street, Pittsburgh, PA 15213-3180.

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Lane Mercer, M.D.
Head, Section of Gynecology
Northwestern University Medical School
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Chicago, Illinois 60611
(312) 908-4868

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Perinatologist

The Department of Obstetrics and Gynecology of the Long Island Jewish Medical Center is recruiting a third Perinatologist to join the Division of Maternal/Fetal Medicine. The Medical Center is an 825-bed institution with approximately 4,000 deliveries each year, 40% of which are "high risk". An established Antepartum Testing Unit operated by the Division offers sophisticated ultrasonographic facilities and doppler technologies for fetal blood flow assessment. Our Neonatal ICU is one of the largest and most advanced in the entire metropolitan area. Experience with invasive techniques like CVS and PUBS preferred.

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Murray, UT 84157-0010
or call toll-free, 1-800-888-3134

THE DEPARTMENT OF OBSTETRICS AND GYNECOLOGY UNIVERSITY OF WISCONSIN MEDICAL SCHOOL

is seeking applicants for two (2) additional full-time faculty positions in MATERNAL FETAL MEDICINE for a new women's health center and academic program at its Milwaukee Clinical Campus. The current faculty includes two perinatologists.

Sinai Samaritan Medical Center has recently consolidated its obstetrical and gynecological services in a redesigned facility in downtown Milwaukee. The department has the largest perinatal service in Wisconsin (5500 deliveries) and has a dedicated antenatal testing and perinatal ultrasonography unit, a midwifery, and adolescent pregnancy service. The department has an affiliation with St. Luke's Medical Center in Milwaukee for its residency program.

Qualifications include board candidacy or certification in maternal-fetal medicine and a commitment to teaching residents, medical students, and clinical research. Competitive salary package. Contact:

Chester B. Martin, M.D.
Chairman
Department of Obstetrics/Gynecology
University of Wisconsin Medical School
600 Highland Avenue, H4/654 CSC
Madison, WI 53792

Fredrik F. Broekhuizen, M.D.
Chairman and Chief
Milwaukee Campus
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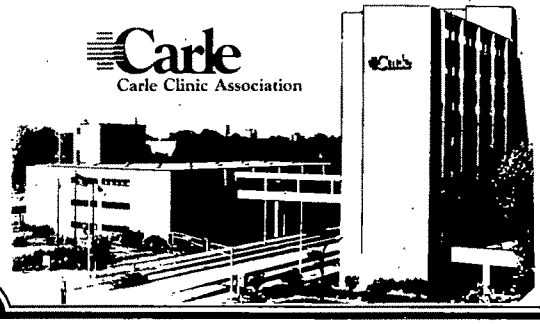
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Robert C. Parker, Jr., MD
Medical Director
Carle Clinic Association
602 W. University, Urbana, Illinois 61801
Phone 217-383-3399 - Fax 217-383-3163

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Applications are invited for the Hartford Foundation/AFAR Fellowship Program in Geriatric Gynecology.

The program, funded by the *John A. Hartford Foundation of New York City* and administered by the American Federation for Aging Research (AFAR), is designed to develop a cadre of gynecologists committed to an academic career with specialized training in the geriatric aspects of their specialties.

Two Fellowships will be awarded in 1992, each offering a grant of $50,000 annually, for the two-year period beginning July 1, 1993.

Applicants must be affiliated with or must plan to pursue their fellowship at one of the institutions which participate in the Hartford Foundation's Academic Geriatric Recruitment Initiative. These institutions are: Bowman Gray School of Medicine, Case Western Reserve University, Duke University, Harvard University, Johns Hopkins University, Mount Sinai School of Medicine, Stanford University, St. Louis University, University of California at Los Angeles, University of Connecticut, University of Michigan, University of Pennsylvania, and University of Washington.

Applications must be filed by an institution on behalf of an individual candidate and must include a statement of plans for the development of curriculum materials related to geriatric gynecology. The application must bear the endorsement of institutional mentors representing the gynecology and geriatric departments or divisions.

Applicants must be board certified or eligible by July 1, 1993 and need not have had previous training in geriatric medicine or gerontology.

Applications must be accompanied by letters of reference from three individuals who know the candidate and by the *curricula vitae* of the candidate and the designated mentors. The deadline for the submission of applications is September 30, 1992. Awards will be announced by October 31, 1992.

Applications and further information may be secured from:

American Federation for Aging Research (AFAR)
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New York, NY 10021
(212) 570-2090 Fax: (212) 570-2496

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4. Did you know that low birthweight is associated with a greater risk of infant death and mental and physical handicaps? ☐ YES ☐ NO

5. Did you know that heroin use during pregnancy increases the likelihood of stillbirth and death among newborns? ☐ YES ☐ NO

6. Did you know that if you or any of your sexual partners ever used an illicit IV drug, you may be infected with the AIDS virus (HIV), and that you can pass that virus on to your unborn child? ☐ YES ☐ NO

7. Did you know that if you use cocaine, are addicted to heroin, or are methadone-dependent, your children will be at a greater risk for sudden infant death syndrome (SIDS)? ☐ YES ☐ NO

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9. Is your doctor aware of all the prescription and/or illicit drugs you may be taking? ☐ YES ☐ NO

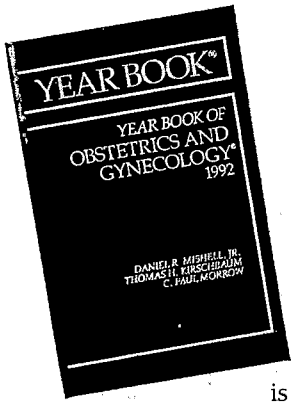
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INDICATIONS AND USAGE: PREVENTION OF PREGNANCY.

CONTRAINDICATIONS: Oral contraceptives should not be used in women who currently have the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophlebitis or thromboembolic disorders. 3. Cerebral vascular or coronary artery disease. 4. Known or suspected carcinoma of the breast. 5. Carcinoma of the endometrium or other known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Cholestatic jaundice of pregnancy or jaundice with prior pill use. 8. Hepatic adenomas or carcinomas. 9. Known or suspected pregnancy.

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risks of several serious conditions including myocardial infarction, thromboembolism, stroke, hepatic neoplasia, and gallbladder disease, although the risk of serious morbidity or mortality is very small in healthy women without underlying risk factors. The risk of morbidity and mortality increases significantly in the presence of other underlying risk factors such as hypertension, hyperlipidemias, obesity and diabetes. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks. The information contained in this brief summary is principally based on studies carried out in patients who used oral contraceptives with higher formulations of estrogens and progestogens than those in common use today. The effect of long term use of the oral contraceptives with lower formulations of both estrogens and progestogens remains to be determined. Throughout this brief summary epidemiological studies reported are of two types: retrospective or case control studies and prospective or cohort studies. Case control studies provide a measure of the relative risk of a disease, namely a *ratio* of the incidence of a disease among oral contraceptive users to that among nonusers. The relative risk does not provide information on the actual clinical occurrence of a disease. Cohort studies provide a measure of attributable risk, which is the *difference* in the incidence of disease between oral contraceptive users and nonusers. The attributable risk does provide information about the actual occurrence of a disease in the population. For further information, the reader is referred to a text on epidemiological methods. 1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS. a. Myocardial Infarction. An increased risk of myocardial infarction has been associated with oral contraceptive use. This risk is primarily in smokers or women with other underlying risk factors for coronary artery disease such as hypertension, hypercholesterolemia, morbid obesity, and diabetes. The relative risk of heart attack for current oral contraceptive users has been estimated to be two to six. The risk is very low under the age of 30. Smoking in combination with oral contraceptive use has been shown to contribute substantially to the incidence of myocardial infarctions in women in their mid-thirties or older with smoking accounting for the majority of excess cases. Mortality rates associated with circulatory disease have been shown to increase substantially in smokers, especially in those 35 years of age and older among women who use oral contraceptives. Oral contraceptives may compound the effects of well-known risk factors, such as hypertension, diabetes, hyperlipidemias, age and obesity. In particular, some progestogens are known to decrease HDL cholesterol and cause glucose intolerance, while estrogens may create a state of hyperinsulinism. Oral contraceptives have been shown to increase blood pressure among users (see section 9 in WARNINGS). Similar effects on risk factors have been associated with an increased risk of heart disease. Oral contraceptives must be used with caution in women with cardiovascular disease risk factors. b. Thromboembolism. An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Case control studies have found the relative risk of users compared to non-users to be 3 for the first episode of superficial venous thrombosis, 4 to 11 for deep vein thrombosis or pulmonary embolism, and 1.5 to 6 for women with predisposing conditions for venous thromboembolic disease. Cohort studies have shown the relative risk to be somewhat lower, about 3 for new cases and about 4.5 for new cases requiring hospitalization. The risk of thromboembolic disease associated with oral contraceptives is not related to length of use and disappears after pill use is stopped. A two- to four-fold increase in relative risk of post-operative thromboembolic complications has been reported with the use of oral contraceptives. The relative risk of venous thrombosis in women who have predisposing conditions is twice that of women without such medical conditions. If feasible, oral contraceptives should be discontinued at least four weeks prior to and for two weeks after elective surgery of a type associated with an increase in risk of thromboembolism and during and following prolonged immobilization. Since the immediate postpartum period is also associated with an increased risk of thromboembolism, oral contraceptives should be started no earlier than four weeks after delivery in women who elect not to breast feed. c. Cerebrovascular diseases. Oral contraceptives have been shown to increase both the relative and attributable risks of cerebrovascular events (thrombotic and hemorrhagic strokes), although, in general, the risk is greatest among older (>35 years), hypertensive women who also smoke. Hypertension was found to be a risk factor for both users and non-users, for both types of strokes, and smoking interacted to increase the risk of stroke. In a large study, the relative risk of thrombotic strokes has been shown to range from 3 for normotensive users to 14 for users with severe hypertension. The relative risk of hemorrhagic stroke is reported to be 1.2 for non-smokers who used oral contraceptives, 2.6 for smokers who did not use oral contraceptives, 7.6 for smokers who used oral contraceptives, 1.8 for normotensive users and 25.7 for users with severe hypertension. The attributable risk is also greater in older women. d. Dose-related risk of vascular disease from oral contraceptives. A positive association has been observed between the amount of estrogen and progestogen in oral contraceptives and the risk of vascular disease. A decline in serum high density lipoproteins (HDL) has been reported with many progestational agents. A decline in serum high density lipoproteins has been associated with an increased incidence of ischemic heart disease. Because estrogens increase HDL cholesterol, the net effect of an oral contraceptive depends on a balance achieved between doses of estrogen and progestogen and the activity of the progestogen used in the contraceptive. The activity and amount of both hormones should be considered in the choice of an oral contraceptive. Minimizing exposure to estrogen and progestogen is in keeping with good principles of therapeutics. For any particular estrogen/progestogen combination, the dosage regimen prescribed should be one which contains the least amount of estrogen and progestogen that is compatible with a low failure rate and the needs of the individual patient. New acceptors of oral contraceptive agents should be started on preparations containing 0.035 mg or less of estrogen. e. Persistence of risk of vascular disease. There are two studies which have shown persistence of risk of vascular disease for ever-users of oral contraceptives. In a study in the United States, the risk of developing myocardial infarction after discontinuing oral contraceptives persists for at least 9 years for women 40-49 years who had used oral contraceptives for five or more years, but this increased risk was not demonstrated in other age groups. In another study in Great Britain, the risk of developing cerebrovascular disease persisted for at least 6 years after discontinuation of oral contraceptives, although excess risk was very small. However, both studies were performed with oral contraceptive formulations containing 50 micrograms or higher of estrogens. 2. ESTIMATES OF MORTALITY FROM CONTRACEPTIVE USE. One study gathered data from a variety of sources which have estimated the mortality rate associated with different methods of contraception at different ages. These estimates include the combined risk of death associated with contraceptive methods plus the risk attributable to pregnancy in the event of method failure. Each method of contraception has its specific benefits and risks. The study concluded that with the exception of oral contraceptive users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of birth control is low and below that associated with childbirth. However, smokers 35 and older and non-smokers 40 and older who use oral contraceptives have a significant increase in mortality higher than those using other methods of birth control. These facts must be weighed in conjunction with failure rates for other methods and the risk associated with subsequent pregnancy. 3. CARCINOMA OF THE REPRODUCTIVE ORGANS. Numerous epidemiological studies have been performed on the incidence of breast, endometrial, ovarian and cervical cancer in women using oral contraceptives. While there are conflicting reports most studies suggest that use of oral contraceptives is not associated with an overall increase in the risk of developing breast cancer. Some studies have reported an increased relative risk of developing breast cancer, particularly at a younger age. This increased relative risk appears to be related to duration of use. Some studies suggest that oral contraceptive use has been associated with an increase in the risk of cervical intraepithelial neoplasia in some populations of women. However, there continues to be controversy about the extent to which such findings may be due to differences in sexual behavior and other factors. 4. HEPATIC NEOPLASIA. Benign hepatic adenomas are associated with oral contraceptive use, although the incidence of benign tumors is rare in the United States. Indirect calculations have estimated the attributable risk to be in the range of 3.3 cases/100,000 for users, a risk that increases after four or more years of use especially with oral contraceptives of higher dose. Rupture of benign, hepatic adenomas may cause death through intra-abdominal hemorrhage. Studies from Britain have shown an increased risk of developing hepatocellular carcinoma in long-term (>8 years) oral contraceptive users. However, these cancers are rare in the U.S. and the attributable risk (the excess incidence) of liver cancers in oral contraceptive users approaches less than one per million users. 5. OCULAR LESIONS. There have been clinical case reports of retinal thrombosis associated with the use of oral contraceptives. Oral contraceptives should be discontinued if there is unexplained partial or complete loss of vision; onset of proptosis or diplopia, papilledema, or retinal vascular lesions. Appropriate diagnostic and therapeutic measures should be undertaken immediately. 6. ORAL CONTRACEPTIVE USE BEFORE OR DURING EARLY PREGNANCY. Extensive epidemiological studies have revealed no increased risk of birth defects in women who have used oral contraceptives prior to pregnancy. The majority of recent studies also do not indicate a teratogenic effect, particularly in so far as cardiac anomalies and limb reduction defects are concerned, when taken inadvertently during early pregnancy. The administration of oral contraceptives to induce withdrawal bleeding should not be used as a test for pregnancy. Oral contraceptives should not be used during pregnancy to treat threatened or habitual abortion. It is recommended that for any patient who has missed two consecutive periods pregnancy should be ruled out before continuing oral contraceptive use. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period. Oral contraceptive use should be discontinued until pregnancy is ruled out. 7. GALLBLADDER DISEASE. Earlier studies have reported an increased lifetime relative risk of gallbladder surgery in users of oral contraceptives and estrogens. More recent studies, however, have shown that the relative risk of developing gallbladder disease among oral contraceptive users may be minimal. The recent findings of minimal risk may be related to the use of oral contraceptive formulations containing lower hormonal doses of estrogens and progestogens. 8. CARBOHYDRATE AND LIPID METABOLIC EFFECTS. Oral contraceptives have been shown to cause a decrease in glucose tolerance in a significant percentage of users. This effect has been shown to be directly related to estrogen dose. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents. However, in the non-diabetic woman, oral contraceptives appear to have no effect on fasting blood glucose. Because of these demonstrated effects prediabetic and diabetic women in particular should be carefully monitored while taking oral contraceptives. A small proportion of women will have persistent hypertriglyceridemia while on the pill. As discussed earlier (see WARNINGS 1a and 1d), changes in serum triglycerides and lipoprotein levels have been reported in oral contraceptive users. 9. ELEVATED BLOOD PRESSURE. An increase in blood pressure has been reported in women taking oral contraceptives, and this increase is more likely in older oral contraceptive users and with extended duration of use. Data from the Royal College of General Practitioners and subsequent randomized trials have shown that the incidence of hypertension increases with increasing progestational activity. Women with a history of hypertension or hypertension-related diseases, or renal disease, should be encouraged to use another method of contraception. If women elect to use oral contraceptives, they should be monitored closely and if significant elevation of blood pressure occurs, oral contraceptives should be discontinued. For most women, elevated blood pressure will return to normal after stopping oral contraceptives, and there is no difference in the occurrence of hypertension between former and never users. 10. HEADACHE. The onset or exacerbation of migraine or development of headache with a new pattern which is recurrent, persistent or severe requires discontinuation of oral contraceptives and evaluation of the cause. 11. BLEEDING IRREGULARITIES. Breakthrough bleeding and spotting are sometimes encountered in patients on oral contraceptives, especially during the first three months of use. Non-hormonal causes should be considered and adequate diagnostic measures taken to rule out malignancy or pregnancy in the event of breakthrough bleeding as in the case of any abnormal vaginal bleeding. If pathology has been excluded, time or a change to another formulation may solve the problem. In the event of amenorrhea, pregnancy should be ruled out. Some women may encounter post-pill amenorrhea or oligomenorrhea, especially when such a condition was preexistent. 12. ECTOPIC PREGNANCY. Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. **PRECAUTIONS:** 1. PHYSICAL EXAMINATION AND FOLLOW UP. A complete medical history and physical examination should be taken prior to the initiation or reinstitution of oral contraceptives and at least annually during use of oral contraceptives. These physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including cervical cytology, and relevant laboratory tests. In case of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be conducted to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care. 2. LIPID DISORDERS. Women who are being treated for hyperlipidemias should be followed closely if they elect to use oral contraceptives. Some progestogens may elevate LDL levels and may render the control of hyperlipidemias more difficult. 3. LIVER FUNCTION. If jaundice develops in any woman receiving such drugs, the medication should be discontinued. Steroid hormones may be poorly metabolized in patients with impaired liver function. 4. FLUID RETENTION. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention. 5. EMOTIONAL DISORDERS. Women with a history of depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. 6. CONTACT LENSES. Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist. 7. DRUG INTERACTIONS. Reduced efficacy and increased incidence of breakthrough bleeding and menstrual irregularities have been associated with concomitant use of rifampin. A similar association, though less marked, has been suggested with barbiturates, phenylbutazone, phenytoin sodium, and possibly with griseofulvin, ampicillin and tetracyclines. 8. INTERACTIONS WITH LABORATORY TESTS. Certain endocrine and liver function tests and blood components may be affected by oral contraceptives: a. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3, increased norepinephrine-induced platelet aggregability. b. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or by radio-immunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. c. Other binding proteins may be elevated in serum. d. Sex-binding globulins are increased and result in elevated levels of total circulating sex steroids and corticoids; however, free or biologically active levels remain unchanged. e. Triglycerides may be increased. f. Glucose tolerance may be decreased. g. Serum folate levels may be depressed by oral contraceptive therapy. This may be of clinical significance if a woman becomes pregnant shortly after discontinuing oral contraceptives. 9. CARCINOGENESIS. See WARNINGS section. 10. PREGNANCY. Pregnancy Category X. See CONTRAINDICATIONS and WARNINGS sections. 11. NURSING MOTHERS. Small amounts of oral contraceptive steroids have been identified in the milk of nursing mothers and a few adverse effects on the child have been reported, including jaundice and breast enlargement. In addition, oral contraceptives given in the postpartum period may interfere with lactation by decreasing the quantity and quality of breast milk. If possible, the nursing mother should be advised not to use oral contraceptives but to use other forms of contraception until she has completely weaned her child. **INFORMATION FOR THE PATIENT: See Patient Package Insert. ADVERSE REACTIONS:** An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS section). Thrombophlebitis and venous thrombosis with or without embolism. Arterial thromboembolism. Pulmonary embolism. Myocardial infarction. Cerebral hemorrhage. Cerebral thrombosis. Hypertension. Gallbladder disease. Hepatic adenomas or benign liver tumors. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea. Vomiting. Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding. Spotting. Change in menstrual flow. Amenorrhea. Temporary infertility after discontinuation of treatment. Edema. Melasma which may persist. Breast changes: tenderness, enlargement, secretion. Change in weight (increase or decrease). Change in cervical erosion and secretion. Diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives and the association has been neither confirmed nor refuted: Pre-menstrual syndrome. Cataracts. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiform. Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. Hemolytic uremic syndrome. Acne. Changes in libido. Colitis. **OVERDOSAGE:** Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea and withdrawal bleeding may occur in females.

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Serious as well as minor side effects have been reported with the use of oral contraceptives. The physician should remain alert to the earliest manifestations of any symptoms of serious disease and discontinue oral contraceptive therapy when appropriate.

Please see complete Prescribing Information, a brief summary of which is on the preceding page.

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When you start your OC patient with The TRI-NORINYL Total Health Program sample package, you provide the benefits of oral contraception *plus* an exciting new patient-support program.

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Serious as well as minor side effects have been reported following the use of oral contraceptives. These include thrombo-embolic disease.

Please see adjacent page for brief summary of prescribing information.

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TABLETS

TRI-NORINYL®

(NORETHINDRONE AND ETHINYL ESTRADIOL)

Committed to the OB/GYN and women's healthcare

SYNTEX

SYNTEX LABORATORIES, INC.
PALO ALTO, CA 94304

TRI-NORINYL
(NORETHINDRONE AND ETHINYL ESTRADIOL)

ORAL CONTRACEPTIVE AGENTS: BRIEF SUMMARY

DESCRIPTION

TRI-NORINYL 21-DAY Tablets provide an oral contraceptive regimen of 7 blue tablets followed by 9 yellow-green tablets and 5 more blue tablets. Each blue tablet contains norethindrone 0.5 mg and ethinyl estradiol 0.035 mg and each yellow-green tablet contains norethindrone 1 mg and ethinyl estradiol 0.035 mg.

TRI-NORINYL 28-DAY Tablets provide a continuous oral contraceptive regimen of 7 blue tablets, 9 yellow-green tablets, 5 more blue tablets, and then 7 orange tablets. Each blue tablet contains norethindrone 0.5 mg and ethinyl estradiol 0.035 mg, each yellow-green tablet contains norethindrone 1 mg and ethinyl estradiol 0.035 mg, and each orange tablet contains inert ingredients.

INDICATIONS AND USAGE

Oral contraceptives are indicated for the prevention of pregnancy in women who elect to use these products as a method of contraception.

CONTRAINDICATIONS

Oral contraceptives should not be used in women who have the following conditions: Thrombophlebitis or thromboembolic disorders • A past history of deep vein thrombophlebitis or thromboembolic disorders • Cerebral vascular or coronary artery disease • Known or suspected carcinoma of the breast • Carcinoma of the endometrium, and known or suspected estrogen-dependent neoplasia • Undiagnosed abnormal genital bleeding • Cholestatic jaundice of pregnancy or jaundice with prior pill use • Hepatic adenomas, carcinomas or benign liver tumors • Known or suspected pregnancy

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risks of several serious conditions including myocardial infarction, thromboembolism, stroke, hepatic neoplasia and gallbladder disease, although the risk of serious morbidity and mortality increases significantly in the presence of other underlying risk factors such as hypertension, hyperlipidemias, hypercholesterolemia, obesity and diabetes.

Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

The information contained in this package insert is principally based on studies carried out in patients who used oral contraceptives with formulations containing 0.05 mg or higher of estrogen. The effects of long-term use with lower dose formulations of both estrogens and progestogens remain to be determined.

Throughout this labeling, epidemiological studies reported are of two types: retrospective or case control studies and prospective or cohort studies. Case control studies provide a measure of the relative risk of a disease. Relative risk, the *ratio* of the incidence of a disease among oral contraceptive users to that among non-users, cannot be assessed directly from case control studies, but the odds ratio obtained is a measure of relative risk. The relative risk does not provide information on the actual clinical occurrence of a disease. Cohort studies provide not only a measure of the relative risk but a measure of attributable risk, which is the *difference* in the incidence of disease between oral contraceptive users and non-users. The attributable risk does provide information about the actual occurrence of a disease in the population.

1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS

a. Myocardial infarction

An increased risk of myocardial infarction has been attributed to oral contraceptive use. This risk is primarily in smokers or women with other underlying risk factors for coronary artery disease such as hypertension, hypercholesterolemia, morbid obesity and diabetes. The relative risk of heart attack for current oral contraceptive users has been estimated to be 2 to 6. The risk is very low under the age of 30. However, there is the possibility of a risk of cardiovascular disease even in very young women who take oral contraceptives.

Smoking in combination with oral contraceptive use has been shown to contribute substantially to the incidence of myocardial infarctions in women 35 or older, with smoking accounting for the majority of excess cases.

Mortality rates associated with circulatory disease have been shown to increase substantially in smokers over the age of 35 and non-smokers over the age of 40 among women who use oral contraceptives.

Oral contraceptives may compound the effects of well-known risk factors for coronary artery disease, such as hypertension, diabetes, hyperlipidemias, hypercholesterolemia, age and obesity. In particular, some progestogens are known to decrease HDL cholesterol and impair oral glucose tolerance, while estrogens may create a state of hyperinsulinism. Oral contraceptives have been shown to increase blood pressure among users (see **WARNINGS,** section 9). Similar effects on risk factors have been associated with an increased risk of heart disease. Oral contraceptives must be used with caution in women with cardiovascular disease risk factors.

b. Thromboembolism

An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Case control studies have found the relative risk of users compared to non-users to be 3 for the first episode of superficial venous thrombosis, 4.6 to 11 for deep vein thrombosis or pulmonary embolism, and 1.5 to 6 for women with predisposing conditions for venous thromboembolic disease. One cohort study has shown the relative risk to be somewhat lower, about 3 for new cases (subjects with no past history of venous thrombosis or varicose veins) and about 4.5 for new cases requiring hospitalization. The risk of thromboembolic disease due to oral contraceptives is not related to length of use and disappears after pill use is stopped.

A 2- to 6-fold increase in relative risk of post-operative thromboembolic complications has been reported with the use of oral contraceptives. If feasible, oral contraceptives should be discontinued at least 4 weeks prior to and for 2 weeks after elective surgery and during and following prolonged immobilization. Since the immediate postpartum period also is associated with an increased risk of thromboembolism, oral contraceptives should be started no earlier than 4 to 6 weeks after delivery in women who elect not to breast feed.

c. Cerebrovascular diseases

An increase in both the relative and attributable risks of cerebrovascular events (thrombotic and hemorrhagic strokes) has been shown in users of oral contraceptives. In general, the risk is greatest among older (>35 years), hypertensive women who also smoke. Hypertension was found to be a risk factor for both users and non-users for both types of strokes while smoking interacted to increase the risk for hemorrhagic strokes.

In a large study, the relative risk of thrombotic strokes has been shown to range from 3 for normotensive users to 14 for users with severe hypertension. The relative risk of hemorrhagic stroke is reported to be 1.2 for non-smokers who used oral contraceptives, 2.6 for smokers who did not use oral contraceptives, 7.6 for smokers who used oral contraceptives, 1.8 for normotensive users and 25.7 for users with severe hypertension. The attributable risk also is greater in women 35 or older and among smokers.

d. Dose-related risk of vascular disease from oral contraceptives

A positive association has been observed between the amount of estrogen and progestogen in oral contraceptives and the risk of vascular disease. A decline in serum high density lipoproteins (HDL) has been reported with some progestational agents. A decline in serum high density lipoproteins has been associated with an increased incidence of ischemic heart disease. Because estrogens increase HDL cholesterol, the net effect of an oral contraceptive depends on a balance achieved between doses of estrogen and progestogen and the nature and absolute amount of progestogens used in the contraceptives. The amount of both hormones should be considered in the choice of an oral contraceptive.

Minimizing exposure to estrogen and progestogen is in keeping with good principles of therapeutics. For any particular estrogen/progestogen combination, the dosage regimen prescribed should be one which contains the least amount of estrogen and progestogen that is compatible with a low failure rate and the needs of the individual patient. New acceptors of oral contraceptive agents should be started on preparations containing the lowest estrogen content that produces satisfactory results for the individual.

e. Persistence of risk of vascular disease

There are three studies which have shown persistence of risk of vascular disease for ever-users of oral contraceptives. In a study in the United States, the risk of developing myocardial infarction after discontinuing oral contraceptives persists for at least 9 years for women 40–49 years who had used oral contraceptives for 5 or more years, but this increased risk was not demonstrated in other age groups. In another study in Great Britain, the risk of developing cerebrovascular disease persisted for at least 6 years after discontinuation of oral contraceptives, although excess risk was very small. Subarachnoid hemorrhage also has a significantly increased relative risk after termination of use of oral contraceptives. However, these studies were performed with oral contraceptive formulations containing 0.05 mg or higher of estrogen.

2. ESTIMATES OF MORTALITY FROM CONTRACEPTIVE USE

One study gathered data from a variety of sources which have estimated the mortality rates associated with different methods of contraception at different ages. These estimates include the combined risk of death associated with contraceptive methods plus the risk attributable to pregnancy in the event of method failure. Each method of contraception has its specific benefits and risks. The study concluded that with the exception of oral contraceptive users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of birth control is low and below that associated with childbirth. The observation of a possible increase in risk of mortality with age for oral contraceptive users is based on data gathered in the 1970s—but not reported in the U.S. until 1983. However, current clinical practice involves the use of lower estrogen dose formulations combined with careful restriction of oral contraceptive use to women who do not have the various risk factors listed in this labeling.

Because of these changes in practice and, also, because of some limited new data which suggest that the risk of cardiovascular disease with the use of oral contraceptives may now be less than previously observed, the Fertility and Maternal Health Drugs Advisory Committee was asked to review the topic in 1989. The Committee concluded that although cardiovascular disease risks may be increased with oral contraceptive use after age 40 in healthy non-smoking women (even with the newer low-dose formulations), there are greater potential health risks associated with pregnancy in older women and with the alternative surgical and medical procedures which may be necessary if such women do not have access to effective and acceptable means of contraception.

Therefore, the Committee recommended that the benefits of oral contraceptive use by healthy non-smoking women over 40 may outweigh the possible risks. Of course, older women, as all women who take oral contraceptives, should take the lowest possible dose formulation that is effective.

3. CARCINOMA OF THE BREAST AND REPRODUCTIVE ORGANS

Numerous epidemiological studies have been performed on the incidence of breast, endometrial, ovarian and cervical cancer in women using oral contraceptives. The evidence in the literature suggests that use of oral contraceptives is not associated with an increase in the risk of developing breast cancer, regardless of the age and parity of first use or with most of the marketed brands and doses. The Cancer and Steroid Hormone study also showed no latent effect on the risk of breast cancer for at least a decade following long-term use. A few studies have shown a slightly increased relative risk of developing breast cancer, although the methodology of these studies, which included differences in examination of users and non-users and differences in age at start of use, has been questioned. Some studies have reported an increased relative risk of developing breast cancer, particularly at a younger age. This increased relative risk appears to be related to duration of use.

Some studies suggest that oral contraceptive use has been associated with an increase in the risk of cervical intraepithelial neoplasia in some populations of women. However, there continues to be controversy about the extent to which such findings may be due to differences in sexual behavior and other factors.

In spite of many studies of the relationship between oral contraceptive use and breast or cervical cancers, a cause and effect relationship has not been established.

4. HEPATIC NEOPLASIA

Benign hepatic adenomas are associated with oral contraceptive use although the incidence of benign tumors is rare in the United States. Indirect calculations have estimated the attributable risk to be in the range of 3.3 cases per 100,000 for users, a risk that increases after 4 or more years of use. Rupture of rare, benign, hepatic adenomas may cause death through intra-abdominal hemorrhage.

Studies in the United States and Britain have shown an increased risk of developing hepatocellular carcinoma in long-term (>8 years) oral contraceptive users. However, these cancers are extremely rare in the United States and the attributable risk (the excess incidence) of liver cancers in oral contraceptive users is less than 1 per 1,000,000 users.

5. OCULAR LESIONS

There have been clinical case reports of retinal thrombosis associated with the use of oral contraceptives. Oral contraceptives should be discontinued if there is unexplained partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions. Appropriate diagnostic and therapeutic measures should be undertaken immediately.

6. ORAL CONTRACEPTIVE USE BEFORE OR DURING EARLY PREGNANCY

Extensive epidemiological studies have revealed no increased risk of birth defects in women who have used oral contraceptives prior to pregnancy. More recent studies do not suggest a teratogenic effect, particularly insofar as cardiac anomalies and limb reduction defects are concerned, when taken inadvertently during early pregnancy.

The administration of oral contraceptives to induce withdrawal bleeding should not be used as a test for pregnancy. Oral contraceptives should not be used during pregnancy to treat threatened or habitual abortion.

It is recommended that for any patient who has missed 2 consecutive periods, pregnancy should be ruled out before continuing oral contraceptive use. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period. Oral contraceptive use should be discontinued if pregnancy is confirmed.

7. GALLBLADDER DISEASE

Earlier studies have reported an increased lifetime relative risk of gallbladder surgery in users of oral contraceptives and estrogens. More recent studies, however, have shown that the relative risk of developing gallbladder disease among oral contraceptive users may be minimal. The recent findings of minimal risk may be related to the use of oral contraceptive formulations containing lower hormonal doses of estrogens and progestogens.

8. CARBOHYDRATE AND LIPID METABOLIC EFFECTS

Oral contraceptives have been shown to impair oral glucose tolerance. Oral contraceptives containing greater than 0.075 mg of estrogen cause glucose intolerance with impaired insulin secretion, while lower doses of estrogen may produce less glucose intolerance. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents. However, in the non-diabetic woman, oral contraceptives appear to have no effect on fasting blood glucose. Because of these demonstrated effects, prediabetic and diabetic women should be carefully observed while taking oral contraceptives.

Some women may develop persistent hypertriglyceridemia while on the pill. As discussed earlier (see **WARNINGS**, sections 1a. and 1d.), changes in serum triglycerides and lipoprotein levels have been reported in oral contraceptive users.

9. ELEVATED BLOOD PRESSURE

An increase in blood pressure has been reported in women taking oral contraceptives. The incidence of risk also was reported to increase with continued use and among older women. Data from the Royal College of General Practitioners and subsequent randomized trials have shown that the incidence of hypertension increases with increasing concentrations of progestogens.

Women with a history of hypertension or hypertension-related diseases or renal disease should be encouraged to use another method of contraception. If women elect to use oral contraceptives, they should be monitored closely and if significant elevation of blood pressure occurs oral contraceptives should be discontinued. For most women, elevated blood pressure will return to normal after stopping oral contraceptives and there is no difference in the occurrence of hypertension among ever- and never-users.

10. HEADACHE

The onset or exacerbation of migraine or development of headache with a new pattern which is recurrent, persistent or severe requires discontinuation of oral contraceptives and evaluation of the cause.

11. BLEEDING IRREGULARITIES

Breakthrough bleeding and spotting are sometimes encountered in patients on oral contraceptives, especially during the first 3 months of use. Non-hormonal causes should be considered and adequate diagnostic measures taken to rule out malignancy or pregnancy in the event of breakthrough bleeding, as in the case of any abnormal vaginal bleeding. If pathology has been excluded, time or a change to another formulation may solve the problem. In the event of amenorrhea, pregnancy should be ruled out.

Some women may encounter post-pill amenorrhea or oligomenorrhea, especially when such a condition was pre-existent.

PRECAUTIONS

1. PHYSICAL EXAMINATION AND FOLLOW-UP

A complete medical history and physical examination should be taken prior to the initiation or reinstitution of oral contraceptives and at least annually during use of oral contraceptives. These physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including cervical cytology and relevant laboratory tests. In case of undiagnosed, persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be conducted to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care.

2. LIPID DISORDERS

Women who are being treated for hyperlipidemias should be followed closely if they elect to use oral contraceptives. Some progestogens may elevate LDL levels and may render the control of hyperlipidemias more difficult.

3. LIVER FUNCTION

If jaundice develops in any woman receiving oral contraceptives the medication should be discontinued. Steroid hormones may be poorly metabolized in patients with impaired liver function.

4. FLUID RETENTION

Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention.

5. EMOTIONAL DISORDERS

Women with a history of depression should be carefully observed and the drug discontinued if depression recurs to a serious degree.

6. CONTACT LENSES

Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist.

7. DRUG INTERACTIONS

Reduced efficacy and increased incidence of breakthrough bleeding and menstrual irregularities have been associated with concomitant use of rifampin. A similar association though less marked, has been suggested with barbiturates, phenylbutazone, phenytoin sodium, and possibly with griseofulvin, ampicillin and tetracyclines.

8. INTERACTIONS WITH LABORATORY TESTS

Certain endocrine and liver function tests and blood components may be affected by oral contraceptives:

a. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.

b. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG. Free T4 concentration is unaltered.

c. Other binding proteins may be elevated in serum.

d. Sex steroid binding globulins are increased and result in elevated levels of total circulating sex steroids and corticoids; however, free or biologically active levels remain unchanged.

e. Triglycerides may be increased.

f. Glucose tolerance may be decreased.

g. Serum folate levels may be depressed by oral contraceptive therapy. This may be of clinical significance if a woman becomes pregnant shortly after discontinuing oral contraceptives.

9. CARCINOGENESIS

See **WARNINGS** section.

10. PREGNANCY

Pregnancy Category X. See **CONTRAINDICATIONS** and **WARNINGS** sections.

11. NURSING MOTHERS

Small amounts of oral contraceptive steroids have been identified in the milk of nursing mothers and a few adverse effects on the child have been reported, including jaundice and breast enlargement. In addition, oral contraceptives given in the postpartum period may interfere with lactation by decreasing the quantity and quality of breast milk. If possible, the nursing mother should be advised not to use oral contraceptives but to use other forms of contraception until she has completely weaned her child.

INFORMATION FOR THE PATIENT

See **PATIENT LABELING.**

ADVERSE REACTIONS

An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see **WARNINGS** section): Thrombophlebitis • Arterial thromboembolism • Pulmonary embolism • Myocardial infarction • Cerebral hemorrhage • Cerebral thrombosis • Hypertension • Gallbladder disease • Hepatic adenomas, carcinomas or benign liver tumors

There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: Mesenteric thrombosis • Retinal thrombosis

The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea • Vomiting • Gastrointestinal symptoms (such as abdominal cramps and bloating) • Breakthrough bleeding • Spotting • Change in menstrual flow • Amenorrhea • Temporary infertility after discontinuation of treatment • Edema • Melasma which may persist • Breast changes: tenderness, enlargement, secretion • Change in weight (increase or decrease) • Change in cervical erosion and secretion • Diminution in lactation when given immediately postpartum • Cholestatic jaundice • Migraine • Rash (allergic) • Mental depression • Reduced tolerance to carbohydrates • Vaginal candidiasis • Change in corneal curvature (steepening) • Intolerance to contact lenses

The following adverse reactions have been reported in users of oral contraceptives and the association has been neither confirmed nor refuted: Pre-menstrual syndrome • Cataracts • Changes in appetite • Cystitis-like syndrome • Headache • Nervousness • Dizziness • Hirsutism • Loss of scalp hair • Erythema multiforme • Erythema nodosum • Hemorrhagic eruption • Vaginitis • Porphyria • Impaired renal function • Hemolytic uremic syndrome • Budd-Chiari syndrome • Acne • Changes in libido • Colitis

OVERDOSAGE

Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

NON-CONTRACEPTIVE HEALTH BENEFITS

The following non-contraceptive health benefits related to the use of oral contraceptives are supported by epidemiological studies which largely utilized oral contraceptive formulations containing estrogen doses exceeding 0.035 mg of ethinyl estradiol or 0.05 mg of mestranol.

Effects on menses: • Increased menstrual cycle regularity • Decreased blood loss and decreased incidence of iron deficiency anemia • Decreased incidence of dysmenorrhea

Effects related to inhibition of ovulation: • Decreased incidence of functional ovarian cysts • Decreased incidence of ectopic pregnancies

Effects from long-term use: • Decreased incidence of fibroadenomas and fibrocystic disease of the breast • Decreased incidence of acute pelvic inflammatory disease • Decreased incidence of endometrial cancer • Decreased incidence of ovarian cancer

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American Journal of Obstetrics and Gynecology

Founded in 1920

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Richmond, Virginia

Before the luteinizing hormone surge, pituitary luteinizing hormone-β messenger ribonucleic acid expression, but not luteinizing hormone content, is decreased in middle-aged rats with attenuated luteinizing hormone surges.

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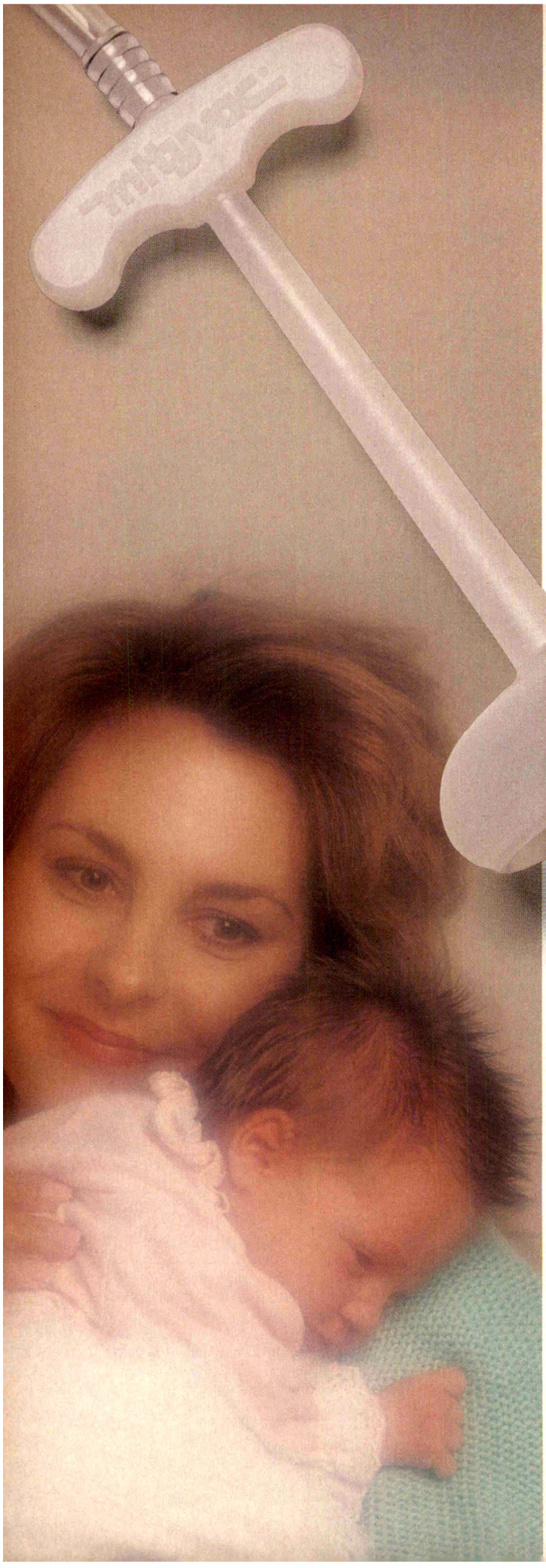
G. William Bates, MD, G. Martin French, MD, Betty B. Humphries, MN, and Dawn W. Blackhurst, MS

Greenville, South Carolina

Seventeen (41.5%) of 41 women with a dehydroepiandrosterone sulfate value >2.8 μg/ml had a positive corticotropin stimulation test result; 50% of those with a positive test result conceived within 6 months of receiving prednisone suppression therapy.

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INDICATIONS AND USAGE
TREATMENT

CEFOTAN is indicated for the therapeutic treatment of the following infections when caused by susceptible strains of the designated organisms:

Urinary Tract Infections caused by *E. coli, Klebsiella* species (including *K. pneumoniae*), *Proteus mirabilis,* and *Proteus* sp (which may include the organisms now called *Proteus vulgaris, Providencia rettgeri,* and *Morganella morganii*).

Lower Respiratory Tract Infections caused by *Streptococcus pneumoniae* (formerly *D. pneumoniae*), *Staphylococcus aureus* (penicillinase- and nonpenicillinase-producing strains), *Haemophilus influenzae* (including ampicillin-resistant strains), *Klebsiella* species (including *K. pneumoniae*), *E. coli, Proteus mirabilis,* and *Serratia marcescens**.

Skin and Skin Structure Infections due to *Staphylococcus aureus* (penicillinase- and nonpenicillinase-producing strains), *Staphylococcus epidermidis, Streptococcus pyogenes, Streptococcus* species (excluding enterococci), *Escherichia coli, Klebsiella pneumoniae, Peptococcus niger**, *Peptostreptococcus* species.

Gynecologic Infections caused by *Staphylococcus aureus* (including penicillinase- and nonpenicillinase-producing strains), *Staphylococcus epidermidis, Streptococcus* species (excluding enterococci), *Streptococcus agalactiae, E. coli, Proteus mirabilis, Neisseria gonorrhoeae,* Bacteroides species (excluding *B. distasonis, B. ovatus, B. thetaiotaomicron*), *Fusobacterium* species*, and gram-positive anaerobic cocci (including *Peptococcus niger* and *Peptostreptococcus* species).

Cefotetan, like other cephalosporins, has no activity against *Chlamydia trachomatis.* Therefore, when cephalosporins are used in the treatment of pelvic inflammatory disease, and *C. trachomatis* is one of the suspected pathogens, appropriate antichlamydial coverage should be added.

Intra-abdominal Infections caused by *E. coli, Klebsiella* species (including *K. pneumoniae*), *Streptococcus* species (excluding enterococci), *Bacteroides* species (excluding *B. distasonis, B. ovatus, B. thetaiotaomicron*) and *Clostridium* species*.

Bone and Joint Infections caused by *Staphylococcus aureus**.

*Efficacy for this organism in this organ system was studied in fewer than ten infections.

Specimens for bacteriological examination should be obtained in order to isolate and identify causative organisms and to determine their susceptibilities to cefotetan. Therapy may be instituted before results of susceptibility studies are known; however, once these results become available, the antibiotic treatment should be adjusted accordingly.

In cases of confirmed or suspected gram-positive or gram-negative sepsis or in patients with other serious infections in which the causative organism has not been identified, it is possible to use CEFOTAN concomitantly with an aminoglycoside. Cefotetan combinations with aminoglycosides have been shown to be synergistic *in vitro* against many Enterobacteriaceae and also some other gram-negative bacteria. The dosage recommended in the labeling of both antibiotics may be given and depends on the severity of the infection and the patient's condition.

NOTE: If CEFOTAN and an aminoglycoside are used concomitantly, renal function should be carefully monitored, especially if higher dosages of the aminoglycoside are to be administered or if therapy is prolonged, because of the potential nephrotoxicity and ototoxicity of aminoglycosidic antibiotics. Although, to date, nephrotoxicity has not been noted when CEFOTAN was given alone, it is possible that nephrotoxicity may be potentiated if CEFOTAN is used concomitantly with an aminoglycoside.

PROPHYLAXIS

The preoperative administration of CEFOTAN may reduce the incidence of certain postoperative infections in patients undergoing surgical procedures that are classified as clean contaminated or potentially contaminated (e.g., cesarean section, abdominal or vaginal hysterectomy, transurethral surgery, biliary tract surgery, and gastrointestinal surgery).

The prophylactic dose of CEFOTAN should be administered 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, CEFOTAN should be administered intravenously after the clamping of the umbilical cord.

If there are signs and symptoms of infection, specimens for culture should be obtained for identification of the causative organism so that appropriate therapeutic measures may be initiated.

CONTRAINDICATIONS

CEFOTAN is contraindicated in patients with known allergy to the cephalosporin group of antibiotics.

WARNINGS

BEFORE THERAPY WITH CEFOTAN IS INSTITUTED, CAREFUL INQUIRY SHOULD BE MADE TO DETERMINE WHETHER THE PATIENT HAS HAD PREVIOUS HYPERSENSITIVITY REACTIONS TO CEFOTETAN DISODIUM, CEPHALOSPORINS, PENICILLINS, OR OTHER DRUGS. IF THIS PRODUCT IS TO BE GIVEN TO PENICILLIN-SENSITIVE PATIENTS, CAUTION SHOULD BE EXERCISED BECAUSE CROSS-HYPERSENSITIVITY AMONG BETA-LACTAM ANTIBIOTICS HAS BEEN CLEARLY DOCUMENTED AND MAY OCCUR IN UP TO 10% OF PATIENTS WITH A HISTORY OF PENICILLIN ALLERGY. IF AN ALLERGIC REACTION TO CEFOTAN OCCURS, DISCONTINUE THE DRUG. SERIOUS ACUTE HYPERSENSITIVITY REACTIONS MAY REQUIRE TREATMENT WITH EPINEPHRINE AND OTHER EMERGENCY MEASURES, INCLUDING OXYGEN, INTRAVENOUS FLUIDS, INTRAVENOUS ANTIHISTAMINES, CORTICOSTEROIDS, PRESSOR AMINES AND AIRWAY MANAGEMENT, AS CLINICALLY INDICATED.

Pseudomembranous colitis has been reported with nearly all antibacterial agents, including cefotetan, and may range from mild to life-threatening. Onset of pseudomembranous colitis symptoms may occur during or after antibiotic treatment or surgical prophylaxis. Therefore, it is important to consider this diagnosis in patients who present with diarrhea subsequent to the administration of antibacterial agents.

Treatment with antibacterial agents alters the normal flora of the colon and may permit overgrowth of clostridia. Studies indicate that a toxin produced by *Clostridium difficile* is a primary cause of "antibiotic-associated colitis."

After the diagnosis of pseudomembranous colitis has been established, therapeutic measures should be initiated. Mild cases of pseudomembranous colitis usually respond to discontinuation of the drug alone. In moderate to severe cases, consideration should be given to management with fluids and electrolytes, protein supplementation, and treatment with an oral antibacterial drug effective against *Clostridium difficile.*

In common with many other broad-spectrum antibiotics, CEFOTAN may be associated with a fall in prothrombin activity and, possibly, subsequent bleeding. Those at increased risk include patients with renal or hepatobiliary impairment or poor nutritional state, the elderly, and patients with cancer. Prothrombin time should be monitored and exogenous vitamin K administered as indicated.

PRECAUTIONS

General: As with other broad-spectrum antibiotics, prolonged use of CEFOTAN may result in overgrowth of nonsusceptible organisms. Careful observation of the patient is essential. If superinfection does occur during therapy, appropriate measures should be taken.

CEFOTAN should be used with caution in individuals with a history of gastrointestinal disease, particularly colitis.

Information for Patients: As with some other cephalosporins, a disulfiram-like reaction characterized by flushing, sweating, headache, and tachycardia may occur when alcohol (beer, wine, etc.) is ingested within 72 hours after CEFOTAN administration. Patients should be cautioned about the ingestion of alcoholic beverages following the administration of CEFOTAN.

Drug Interactions: Although to date nephrotoxicity has not been noted when CEFOTAN was given alone, it is possible that nephrotoxicity may be potentiated if CEFOTAN is used concomitantly with an aminoglycoside.

Drug/Laboratory Test Interactions: The administration of CEFOTAN may result in a false-positive reaction for glucose in the urine using Clinitest®†, Benedict's solution, or Fehling's solution. It is recommended that glucose tests based on enzymatic glucose oxidase be used.

As with other cephalosporins, high concentrations of cefotetan may interfere with measurement of serum and urine creatinine levels by Jaffe reaction and produce false increases in the levels of creatinine reported.

Carcinogenesis, Mutagenesis, Impairment of Fertility: Although long-term studies in animals have not been performed to evaluate carcinogenic potential, no mutagenic potential of cefotetan was found in standard laboratory tests.

Cefotetan has adverse effects on the testes of prepubertal rats. Subcutaneous administration of 500 mg/kg/day (approximately 8-16 times the usual adult human dose) on days 6-35 of life (thought to be developmentally analogous to late childhood and prepuberty in humans) resulted in reduced testicular weight and seminiferous tubule degeneration in 10 of 10 animals. Affected cells included spermatogonia and spermatocytes; Sertoli and Leydig cells were unaffected. Incidence and severity of lesions were dose-dependent; at 120 mg/kg/day (approximately 2-4 times the usual human dose) only 1 of 10 treated animals was affected, and the degree of degeneration was mild.

Similar lesions have been observed in experiments of comparable design with other methylthiotetrazole-containing antibiotics and impaired fertility has been reported, particularly at high dose levels. No testicular effects were observed in 7-week-old rats treated with up to 1000 mg/kg/day SC for 5 weeks, or in infant dogs (3 weeks old) that received up to 300 mg/kg/day IV for 5 weeks. The relevance of these findings to humans is unknown.

Pregnancy: Teratogenic Effects. Pregnancy Category B: Reproduction studies have been performed in rats and monkeys at doses up to 20 times the human dose and have revealed no evidence of impaired fertility or harm to the fetus due to cefotetan. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproductive studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Nursing Mothers: Cefotetan is excreted in human milk in very low concentrations. Caution should be exercised when cefotetan is administered to a nursing woman.

Pediatric Use: Safety and effectiveness in children have not been established.

ADVERSE REACTIONS

In clinical studies, the following adverse effects were considered related to CEFOTAN therapy. Those appearing in italics have been reported during postmarketing experience.

Gastrointestinal symptoms occurred in 1.5% of patients; the most frequent were diarrhea (1 in 80) and nausea (1 in 700); *pseudomembranous colitis.* Onset of pseudomembranous colitis symptoms may occur during or after antibiotic treatment or surgical prophylaxis. (See WARNINGS.)

Hematologic laboratory abnormalities occurred in 1.4% of patients and included eosinophilia (1 in 200), positive direct Coombs' test (1 in 250), and thrombocytosis (1 in 300); *agranulocytosis, hemolytic anemia, leukopenia, thrombocytopenia,* and *prolonged prothrombin time.*

Hepatic enzyme elevations occurred in 1.2% of patients and included a rise in SGPT (1 in 150), SGOT (1 in 300), alkaline phosphatase (1 in 700), and LDH (1 in 700).

Hypersensitivity reactions were reported in 1.2% of patients and included rash (1 in 150) and itching (1 in 700); *anaphylactic reactions.*

Local effects were reported in less than 1.0% of patients and included phlebitis at the site of injection (1 in 300), and discomfort (1 in 500).

Miscellaneous: *Fever*

In addition to the adverse reactions listed above which have been observed in patients treated with cefotetan, the following adverse reactions and altered laboratory tests have been reported for cephalosporin-class antibiotics: urticaria, pruritis, Stevens-Johnson syndrome, erythema multiforme, toxic epidermal necrolysis, vomiting, abdominal pain, colitis, superinfection, vaginitis including vaginal candidiasis, renal dysfunction, toxic nephropathy, hepatic dysfunction including cholestasis, aplastic anemia, hemorrhage, increased BUN, increased creatinine, elevated bilirubin, pancytopenia, and neutropenia.

Several cephalosporins have been implicated in triggering seizures, particularly in patients with renal impairment when the dosage was not reduced (see DOSAGE AND ADMINISTRATION and OVERDOSAGE). If seizures associated with drug therapy occur, the drug should be discontinued. Anticonvulsant therapy can be given if clinically indicated.

DOSAGE AND ADMINISTRATION
TREATMENT

The usual adult dosage is 1 or 2 grams of CEFOTAN administered intravenously or intramuscularly every 12 hours for 5 to 10 days. Proper dosage and route of administration should be determined by the condition of the patient, severity of the infection, and susceptibility of the causative organism.

GENERAL GUIDELINES FOR DOSAGE OF CEFOTAN

Type of Infection	Daily Dose	Frequency and Route
Urinary Tract	1-4 grams	500 mg every 12 hours IV or IM 1 or 2 g every 24 hours IV or IM 1 or 2 g every 12 hours IV or IM
Skin & Skin Structure		
Mild-Moderate[a]	2 grams	2 g every 24 hours IV 1 g every 12 hours IV or IM
Severe	4 grams	2 g every 12 hours IV
Other Sites	2-4 grams	1 or 2 g every 12 hours IV or IM
Severe	4 grams	2 g every 12 hours IV
Life-Threatening	6 grams[b]	3 g every 12 hours IV

[a] *K. pneumoniae* skin and skin structure infections should be treated with 1 or 2 grams every 12 hours IV or IM.

[b] Maximum daily dosage should not exceed 6 grams.

If *C. trachomatis* is a suspected pathogen in gynecologic infections, appropriate antichlamydial coverage should be added, since cefotetan has no activity against this organism.

PROPHYLAXIS

To prevent postoperative infection in clean contaminated or potentially contaminated surgery in adults, the recommended dosage is 1 or 2 g of CEFOTAN administered once, intravenously, 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, the dose should be administered as soon as the umbilical cord is clamped.

IMPAIRED RENAL FUNCTION

When renal function is impaired, a reduced dosage schedule must be employed. See FULL PRESCRIBING INFORMATION for a complete description.

NOTE: Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration whenever solution and container permit.

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References: 1. Walker CK, Landers DV, Ohm-Smith MJ, et al. Comparison of cefotetan plus doxycycline with cefoxitin plus doxycycline in the inpatient treatment of acute salpingitis. *Sex Trans Dis.* 1991;18:119-123. **2.** McGregor JA, Gordon SF, Krotec J, Poindexter AN. Results of a randomized, multicenter, comparative trial of a single dose of cefotetan versus multiple doses of cefoxitin as prophylaxis in cesarean section. *Am J Obstet Gynecol.* 1988;158:701-706. **3.** Carver P, Quintiliani R, Nightingale CH. Comparative pharmacokinetic study of cefotetan and cefoxitin in healthy volunteers. *Infect Surg.* April 1986(suppl):11-14. **4.** Ohm-Smith MJ, Sweet RL, Hadley WK. In vitro activity of cefbuperazone and other antimicrobial agents against isolates from the female genital tract. *Antimicrob Agents Chemother.* 1985;27:958-960. **5.** Data on file, Stuart Pharmaceuticals, Wilmington, Delaware. **6.** Souza Dias MB, Jacobus NV, Tally FP, Gorbach SL. Activity of cefotetan against anaerobic bacteria. *Diagn Microbiol Infect Dis.* 1986;4:359-363. **7.** Wexler HM, Finegold SM. In vitro activity of cefotetan compared with that of other antimicrobial agents against anaerobic bacteria. *Antimicrob Agents Chemother.* 1988;32:601-604. **8.** Hennessey TD. Cefotetan: a compilation of susceptibility data of clinical isolates. In: Lode H, Periti P, Strachan CJL, eds. *Cefotetan: A Long-Acting Antibiotic for Polymicrobial Infections.* New York, NY: Churchill Livingstone; 1985:14-22. **9.** Centers for Disease Control. 1989 sexually transmitted diseases treatment guidelines. *MMWR.* 1989;38(suppl S-8):1-43.

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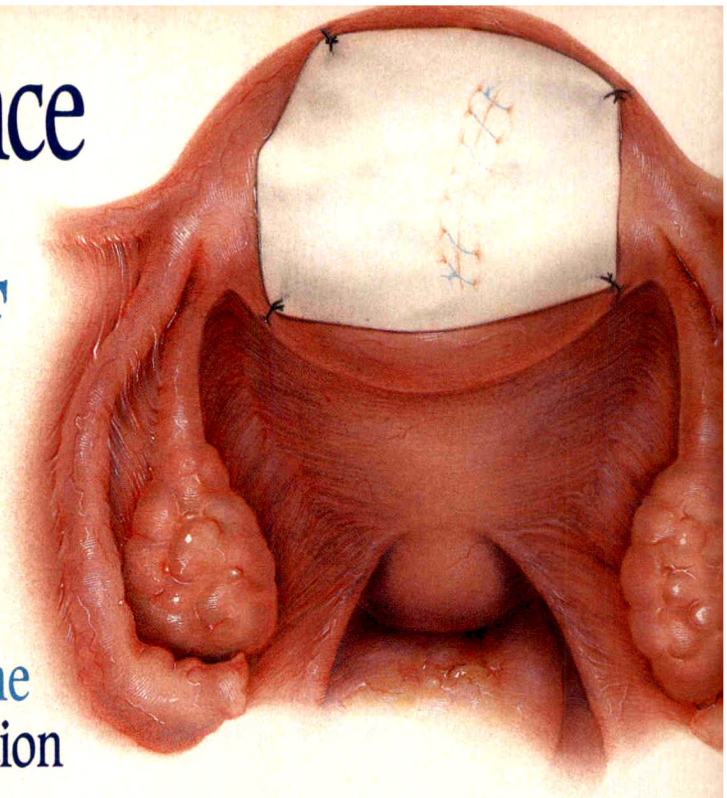
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Invariant Performance in Peritoneal Reconstruction of Myomectomy Sites

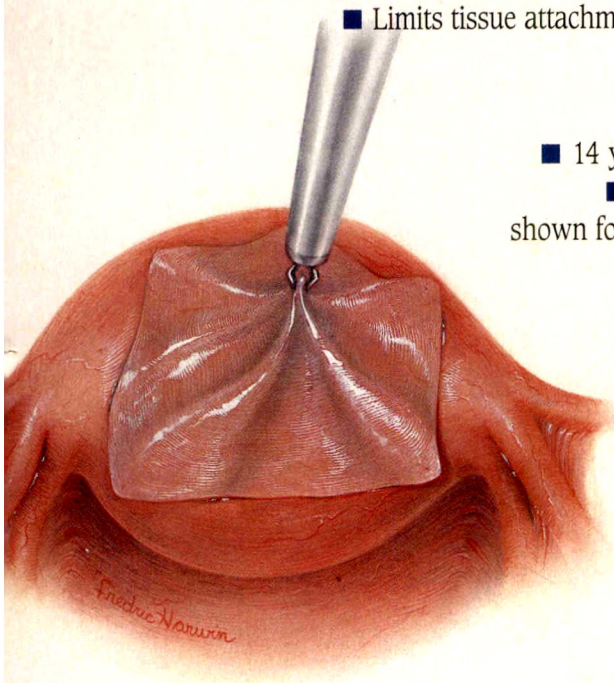


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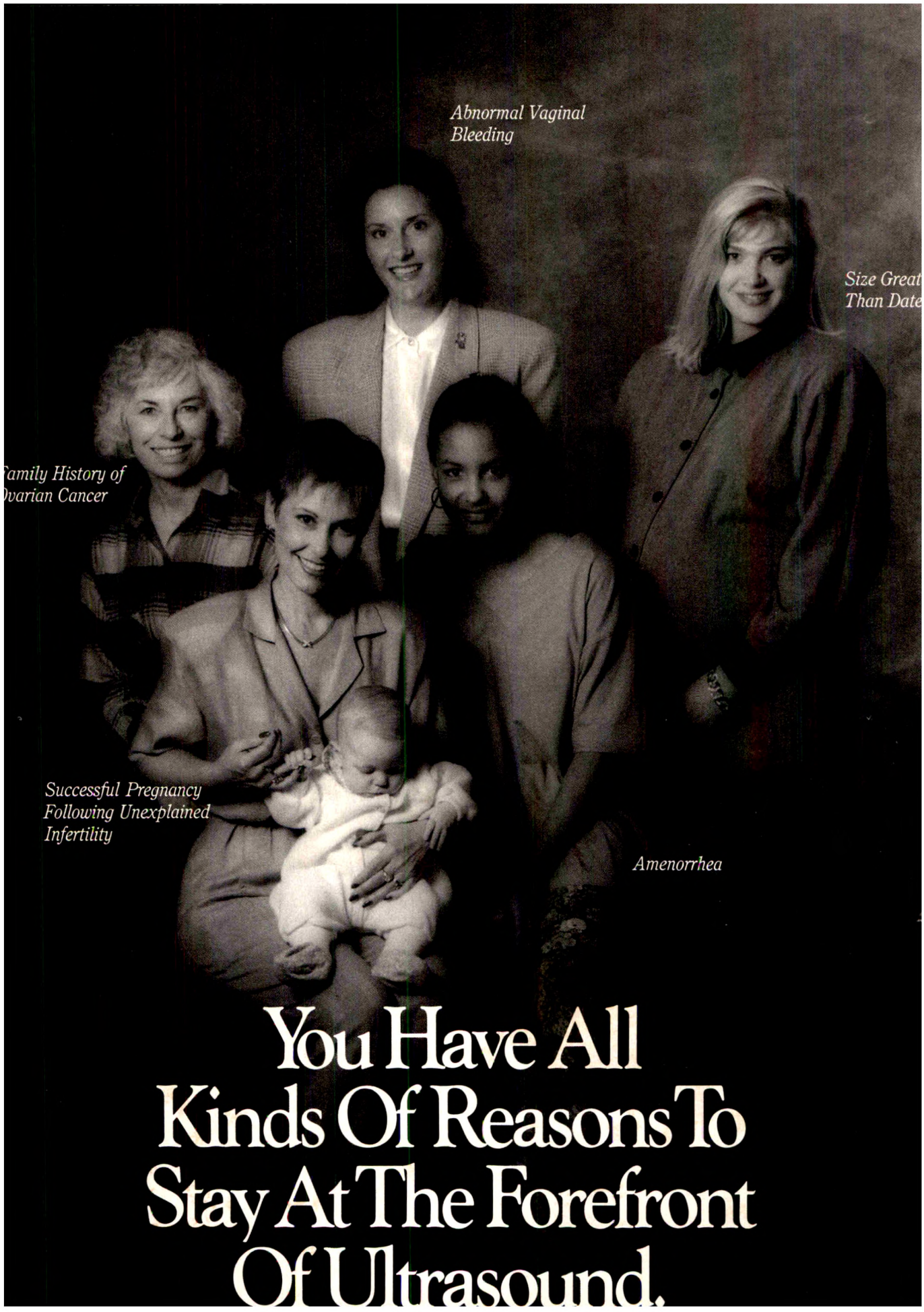
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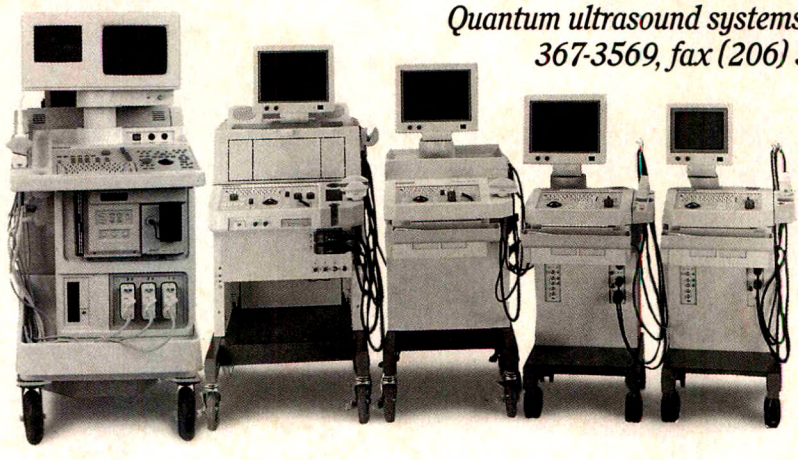
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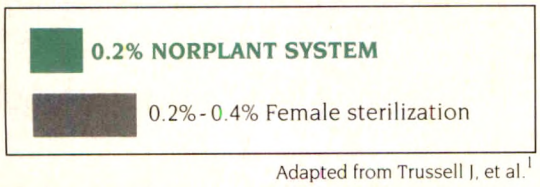


Six reasons why you can feel comfortable recommending it to your patients

Effectiveness comparable to tubal ligation

Although no contraceptive is 100% effective, the NORPLANT SYSTEM is one of the most effective forms of birth control ever developed. The average annual gross pregnancy rate over a five-year period is less than 1%.

Lowest expected and typical failure rates with NORPLANT SYSTEM vs. sterilization during first-year use[1]



Adapted from Trussell J, et al.[1]

Long-term convenience... estrogen-free

NORPLANT SYSTEM, a progestin-only contraceptive, is an excellent option for women desiring a long-term method. Its effectiveness lasts for five years and is not dependent on compliance.

Reversible, with prompt return to previous level of fertility

NORPLANT SYSTEM is reversible at any time; the contraceptive effect ceases when the capsules are removed. In one study, 40% of women wanting to conceive did so within 3 months of removal, 63% by 6 months, 76% by 12 months, and 90% by 24 months.[2]



High patient acceptance despite menstrual pattern changes

NORPLANT SYSTEM causes menstrual irregularities (the most common complaint),* which occur in many women during first-year use and diminish over time. Only 9.1% of patients discontinued during first-year use due to these changes.[3†]

Women who are thoroughly informed of potential menstrual changes and side effects are more tolerant of them when they occur.[4]

Well-received, well-liked by American women

One study of 140 users revealed that 94% were satisfied with NORPLANT SYSTEM, 91% recommended it to their friends, and 74% wanted to use it again.[5]

A 10- to 15-minute office procedure

A local anesthetic and a small 2 mm incision...little or no discomfort during insertion...proper placement should not leave a noticeable scar...in most cases sutures are not needed.

*Serious as well as minor side effects may occur (see prescribing information).

† Based on multicenter trials of 2,470 women.

Please see brief summary of prescribing information on adjacent page.

NORPLANT® SYSTEM
levonorgestrel implants

Lasts 5 years...yet is reversible

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NORPLANT® SYSTEM

levonorgestrel implants

Lasts five years…yet is reversible

BRIEF SUMMARY OF PRESCRIBING INFORMATION. CONSULT THE PACKAGE LITERATURE FOR FULL PRESCRIBING INFORMATION.

Indications and Usage

The NORPLANT SYSTEM is indicated for the prevention of pregnancy and is a long-term (up to 5 years) reversible contraceptive system. The capsules should be removed by the end of the 5th year. New capsules may be inserted at that time if continuing contraceptive protection is desired.

Contraindications

1. Active thrombophlebitis or thromboembolic disorders. 2. Undiagnosed abnormal genital bleeding. 3. Known or suspected pregnancy. 4. Acute liver disease; benign or malignant liver tumors. 5. Known or suspected carcinoma of the breast.

Warnings

A. WARNINGS BASED ON EXPERIENCE WITH THE NORPLANT SYSTEM

1. *Bleeding Irregularities* — Most women can expect some variation in menstrual bleeding patterns. Irregular menstrual bleeding, intermenstrual spotting, prolonged episodes of bleeding and spotting, and amenorrhea may occur, and could mask symptoms of cervical or endometrial cancer. Overall, these irregularities diminish with continued use. Because amenorrhea may occur, missed menstrual periods cannot serve as the only identifier of early pregnancy. Perform pregnancy tests whenever pregnancy is suspected. If pregnancy occurs, the capsules must be removed. Hemoglobin concentrations found in clinical trials generally indicated that reduced menstrual blood loss is associated with NORPLANT SYSTEM use. Blood loss resulting in hemoglobin values consistent with anemia occurred rarely.

2. *Delayed Follicular Atresia* — Atresia of the follicle is sometimes delayed, resulting in enlarged follicles that are clinically indistinguishable from ovarian cysts. In the majority of women, enlarged follicles disappear spontaneously. Rarely, they twist or rupture and surgical intervention may be required.

3. *Ectopic Pregnancies* — Ectopic pregnancies have occurred among NORPLANT SYSTEM users, although clinical studies have shown no increase in the rate of ectopic pregnancies per year among NORPLANT SYSTEM users as compared with users of no method or of IUDs. The incidence among NORPLANT SYSTEM users (1.3 per 1000 woman-years) was significantly below the rate estimated for noncontraceptive users in the U.S. (2.7 to 3.0 per 1000 woman-years). Ectopic pregnancy risk may increase with duration of NORPLANT SYSTEM use and increased weight of the user. Rule out ectopic pregnancy in any patient presenting with lower-abdominal pain.

4. *Breast-feeding* — Steroids are not the contraceptives of first choice for lactating women. Levonorgestrel has been identified in breast milk. Limited data suggests no significant effects on infant growth or health when mothers used the NORPLANT SYSTEM beginning 6 weeks after parturition.

5. *Foreign-body Carcinogenesis* — Rarely, cancers occur at foreign-body intrusion sites or old scars. None has been reported in NORPLANT SYSTEM clinical trials and risk to users is judged to be minimal.

6. *Thromboembolic Disorders* — Remove capsules if active thrombophlebitis or thromboembolic disease develops. With prolonged immobilization removal should be considered.

B. WARNINGS BASED ON EXPERIENCE WITH COMBINATION (PROGESTIN PLUS ESTROGEN) ORAL CONTRACEPTIVES (OCs)

Note: Many of the side effects or risks listed below are thought to be estrogen-related; the association of the NORPLANT SYSTEM progestin-only method to these risks is unknown.

1. *Cigarette Smoking* — Cigarette smoking increases the risk of serious cardiovascular side effects from combined OC use. Risk increases with age and heavy smoking (≥15 cigarettes/day) and is quite marked in women over 35 years old.

2. *Elevated Blood Pressure* — Increase in blood pressure has been reported in combination OC users; prevalence increases with long exposure.

3. *Thromboembolic Disorders and Other Vascular Problems* — An increased risk of thromboembolic and thrombotic disease is associated with combination OC use. Estimate of relative risk is 4- to 11-fold higher for users vs. nonusers.

Cerebrovascular Disorders: Combination OCs increase the relative and attributable risk of cerebrovascular events (thrombotic and hemorrhagic strokes). Generally, risk is greatest among hypertensive women > 35 years of age who smoke.

Myocardial Infarction (MI): An increased risk of MI has been attributed to combined OC use. This is thought to be primarily thrombotic in origin and related to the estrogen component. Increased risk occurs primarily in smokers or women with other underlying risk factors for coronary-artery disease. Relative risk of heart attack for combined OC users is estimated as 2 to 6 times that for nonusers. Absolute risk is very low for women under 30 years old.

Studies indicate a significant trend toward higher MI and stroke rates with increased progestin doses in combination OCs. However, recent data indicated no increased MI risk with past use of levonorgestrel-containing OCs.

4. *Carcinoma* — Recent evidence in the literature suggests no association between OC use and increased risk of breast cancer in the overall population of users. The Cancer and Steroid Hormone (CASH) study also showed no latent effect on breast cancer risk for at least a decade following long-term use. Some of these same studies have shown an increased relative risk of breast cancer in certain subgroups; no consistent pattern has been identified. Some studies suggest an association between combination OCs and an increase in the risk of cervical intra-epithelial neoplasia in some populations of women. The extent to which such findings may be due to differences in sexual behavior and other factors remains controversial. A cause-and-effect relationship between combined OC use and breast or cervical cancer has not been established. Combination OCs may decrease ovarian and endometrial cancer risk. Irregular bleeding patterns associated with NORPLANT SYSTEM use could mask cervical or endometrial cancer symptoms.

5. *Hepatic Tumors* — Hepatic adenomas are associated with combination OC use; estimated incidence is 3 events per 100,000 users per year. Risk increases after 4 or more years of use. Hepatic adenomas are benign but may rupture and cause death through intra-abdominal hemorrhage.

6. *Ocular Lesions* — Retinal thrombosis is associated with OC use and is believed to be related to the estrogen component. However, NORPLANT SYSTEM capsules should be removed if there is unexplained partial or complete vision loss; onset of proptosis or diplopia; papilledema; or retinal vascular lesions. Undertake appropriate diagnostic and therapeutic measures immediately.

7. *Use Before or During Early Pregnancy* — Extensive epidemiological studies reveal no increased risk of birth defects when OCs are used prior to pregnancy. Studies also do not suggest a teratogenic effect when taken inadvertently during early pregnancy. No evidence suggests that risk with NORPLANT SYSTEM use is different.

8. *Gallbladder Disease* — Early studies reported an increased lifetime relative risk of gallbladder surgery in OC or estrogen users. More recent studies, however, indicate that the relative risk of gallbladder disease with OC use may be minimal; this may be related to use of OCs with less estrogen and progestin content.

Precautions

GENERAL

1. *Physical Examination and Follow-up* — A complete medical history and physical examination should be taken prior to implantation or reimplantation of NORPLANT SYSTEM capsules and at least annually during its use. Exams should include special reference to the implant site, blood pressure, breasts, abdomen and pelvic organs, including cervical cytology and relevant laboratory tests. Rule out malignancy in cases of undiagnosed, persistent or recurrent abnormal vaginal bleeding. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care.

2. *Carbohydrate Metabolism* — Altered glucose tolerance is found in some combination and progestin-only OC users. Effects of NORPLANT SYSTEM on carbohydrate metabolism appear minimal. Observe diabetic and prediabetic patients carefully while using the NORPLANT SYSTEM. Follow women being treated for hyperlipidemias closely if using the NORPLANT SYSTEM. Some progestins may elevate LDL and may render control of hyperlipidemias more difficult. (See **Warnings**.)

3. *Liver Function* — Consider removing capsules if jaundice develops. Steroid hormones may be poorly metabolized in patients with impaired liver function.

4. *Fluid Retention* — Steroid contraceptives may cause some degree of fluid retention. Prescribe with caution, and careful monitoring, in patients with conditions possibly aggravated by fluid retention.

5. *Emotional Disorders* — Consider removing capsules if significant depression occurs since the symptom may be drug-related. Observe carefully those with history of depression and consider removal if depression recurs to a serious degree.

6. *Contact Lenses* — Contact-lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist.

7. *Insertion and Removal* — Insertion is advised during the first 7 days of the cycle or immediately following abortion to insure that the woman is not pregnant and to assure contraceptive effectiveness during first cycle of use. Capsules may be inserted at any time during the cycle provided pregnancy has been excluded and a nonhormonal contraceptive method is used for the remainder of the cycle. Insertion is not recommended before 6 weeks postpartum in breast-feeding women. Follow insertion and removal instructions closely. Healthcare professionals are strongly advised to be instructed in the procedures before they attempt them. Proper insertion just under the skin facilitates removals; proper insertion and removal should result in minimal scarring. If all capsules cannot be removed at first attempt, attempt removal later when the site has healed. Bruising may occur at implant site during insertion or removal. Hyperpigmentation may occur over implant site but is usually reversible following removal. **See Full Prescribing Information for Detailed Insertion/Removal Instructions.**

8. *Infections* — Implant site infection has been uncommon (0.7%); aseptic technique and proper insertion/removal reduces possibility of infection. Institute treatment if infection occurs; remove capsules if infection persists.

9. *Expulsion* — Expulsion of capsules was uncommon; frequency increased when capsule placement was extremely shallow, was too close to incision, or when infection was present. Replace expelled capsule with new sterile capsule. Treat and cure any infection before replacement. Contraceptive efficacy may be inadequate with fewer than 6 capsules.

10. *Provisions for Removal* — Advise women that capsules may be removed at any time for any reason. Personnel instructed in removal technique should perform removal on request or at the end of 5 years of usage. Upon removal, dispose of capsules in accordance with Centers for Disease Control Guidelines for biohazardous waste.

DRUG INTERACTIONS: Reduced efficacy (pregnancy) in NORPLANT SYSTEM users has been reported when phenytoin or carbamazepine were used concomitantly. Warn NORPLANT SYSTEM users of possible decreased efficacy with use of related drugs.

DRUG/LABORATORY TEST INTERACTIONS: 1. Sex-hormone-binding globulin concentrations are decreased. 2. Thyroxine concentrations may be slightly decreased and triiodothyronine uptake increased.

CARCINOGENESIS: See **Warnings** section and Full Prescribing Information.

PREGNANCY: Pregnancy Category X. See **Warnings** section and Full Prescribing Information.

NURSING MOTHERS: See **Warnings** section and Full Prescribing Information.

INFORMATION FOR THE PATIENT: See Patient Labeling. Provide copy of patient labeling to the patient. Advise patients that Prescribing Information is available upon request. Inform prospective users of risks and benefits associated with NORPLANT SYSTEM use, with other forms of contraception, with no contraception, and about insertion/removal procedures. Informed consent from all patients may be desired in light of techniques involved with insertion and removal.

Adverse Reactions

The following have been associated with the NORPLANT SYSTEM during first year of use: many bleeding days or prolonged bleeding (27.6%); spotting (17.1%); amenorrhea (9.4%); irregular (onsets of) bleeding (7.6%); frequent bleeding onsets (7.0%); scanty bleeding (5.2%); pain or itching near implant site - usually transient - (3.7%); infection at implant site (0.7%); removal difficulties affecting subjects - based on 849 removals - (6.2%).

Controlled clinical studies suggest that the following, occurring during the first year, are probably associated with NORPLANT SYSTEM use: headache; nervousness; nausea; dizziness; adnexal enlargement; dermatitis; acne; change of appetite; mastalgia; weight gain; hirsutism, hypertrichosis, and scalp-hair loss. The following were reported with a frequency of 5% or greater during the first year and possibly may be related to NORPLANT SYSTEM use: breast discharge; cervicitis; musculoskeletal pain; abdominal discomfort; leukorrhea; vaginitis.

Overdosage

Overdosage may cause fluid retention with its associated effects and uterine bleeding irregularities.

Dosage and Administration

The NORPLANT SYSTEM consists of six Silastic® capsules, each containing 36 mg of the progestin, levonorgestrel. The total administered (implanted) dose is 216 mg. Implantation of all six capsules should be performed during the first 7 days of the onset of menses by a healthcare professional instructed in the NORPLANT SYSTEM insertion technique. Insertion is subdermal in the midportion of the upper arm about 8 to 10 cm above the elbow crease. Distribution should be in a fanlike pattern, about 15 degrees apart, for a total of 75 degrees. Proper insertion will facilitate later removal. **(See Full Prescribing Information for Detailed Insertion/Removal Instructions.)**

CI 4064-1 12/10/90

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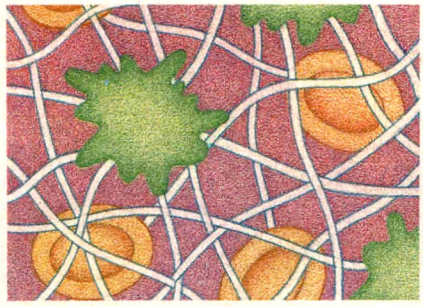
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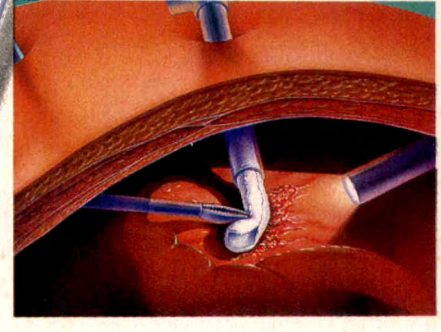
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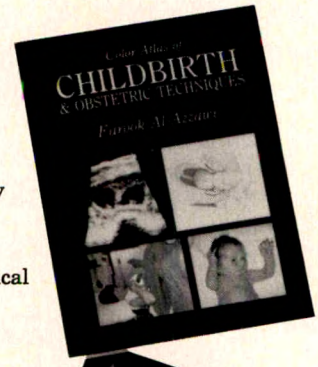
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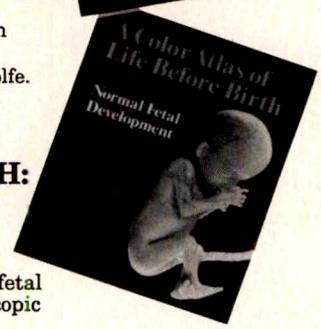
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With the exception of Current Development articles, the length of text material (introduction through *Comment* section) in regular manuscripts accepted for publication normally ranges from 750 to 4200 words (an average of 2000 words). A text of 4200 words or more can seldom be accepted, especially if tables and figures are included. The average manuscript of 2000 words of text with abstract, 3 tables with captions, 2 figures with legends, and references makes a 5.7-page article in the JOURNAL. The 2000 words of text alone makes approximately 8 pages of manuscript typed double-spaced with the required 1-inch margins (approximately 250 words per page). A table or figure that occupies both columns of half a JOURNAL page is equivalent to approximately 500 words in manuscript. Thus, if a greater number of illustrations and tables is used, the length of the text should be adjusted accordingly.

Council of Biology Editors Style Manual or the AMA's *Manual of Style.* Abbreviations in the title are not acceptable. They should be avoided, if possible, in the abstract. In the text they should be kept to a practical minimum. The full term for which an abbreviation stands should precede its first use in the text unless it is a standard unit of measurement.

Either the generic, chemical, or proprietary names of drugs may be used. If the generic or chemical name is used, authors may, if they desire, insert the proprietary name in parentheses after the first mention in the text, with the name of the manufacturer and city and state.

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BOOKS: Ledger WJ. Dystocia and prolonged labor. In: Wilson JR, Carrington ER, eds. Obstetrics and gynecology. St. Louis: CV Mosby, 1987 vol 8:474-93.

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TRANSACTIONS OF THE FIFTY-FOURTH ANNUAL MEETING OF THE SOUTH ATLANTIC ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS

Teenage pregnancy prevention: The vital importance of the medical community's involvement

Presidential address

Willette L. LeHew, MD
Norfolk, Virginia

Prevention is an active process creating conditions and fostering personal attributes that promote the well-being of people. As physicians who are influential in our communities and states, who are experts in the health care field, and who provide primary and specialty care for the majority of female teenagers, we must take a more active advocacy role in teenage pregnancy prevention if we are going to be successful in reducing this devastating medical and social problem.

We all know that adolescent sexuality is complex and that solutions for preventing pregnancies must be multifaceted, and, although each state's efforts are unique, there is clearly a collective sense of what needs to happen in our states to make a difference. Our strategic efforts must include public awareness events, media campaigns, and family life education and health services in our schools, with a strong emphasis on family planning. In all of these we must encourage family-based communication about sexuality. Today I will briefly outline the problems that most of you are aware of and suggest some solutions that I hope you will take back to your community and state to be used in preventive efforts.

Any successful solution in teen pregnancies will also greatly affect and reduce school dropouts, substance abuse, poverty, and crime as these elements increasingly seem to have a synergistic effect. One study[1] estimated that one in four children aged 10 to 17 were at risk for at least one of the following: teenage pregnancy, failing in school, abusing drugs, or becoming delinquent. And at least one in nine were at risk for all of these things happening to them at once. Therefore we need to promote comprehensive, community-based initiatives that help combat these problems and increase young people's community involvement.

We must refocus our main efforts from the futility of providing services to babies already born with birth defects and severe prematurity and providing life-long welfare payments to teen mothers unable to work and live independently to more positive preventive steps. To do this, we must remind ourselves, and convince others, that teen pregnancy continues at epidemic proportions and realize that no mass disorder that has ever affected mankind has been brought under control by attempts to treat already afflicted persons one by one.

We must then focus our energies, thinking, and resources on prevention by concentrating on several general areas. The first is delaying and reducing high-risk sexual behavior. The second is promoting contraception and contraceptive use. The third is using our school systems more efficiently for providing health and education about sexuality. Finally, we must promote programs providing meaningful alternatives to pregnancy. These programs must gain wide support

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from the medical community; local, state, and federal government; businesses; and the church community to be successful. We must also place this issue on the agenda of our state and local medical societies and our specialty societies.

More specific categories of prevention would be to encourage adolescents to abstain from sexual activity, to include more programs involving and targeting teenage boys, to support family life education, to support comprehensive health services in our schools, to encourage greater involvement of individual practitioners with their teenage pregnancies, and to obtain greater availability of contraceptive services for adolescents. After relating a few facts about the scope of the problem, I will discuss several of these specific areas where the medical community should be most influential and effective.

I would encourage each of you to learn your current community and state's statistics on total teen pregnancies, abortions, and live births and their comparison to other areas. Your health department or teen pregnancy council will be happy to supply you with these facts.

One very disturbing fact is that the percentage of teen births outside of marriage increased by 61% nationwide between 1970 and 1988, and in fact in 1988 two thirds of the births to teenagers in this country were illegitimate.[2] For our five states during this period the rate in Virginia increased by 26%, in North Carolina by 34%, in South Carolina by 49%, in Florida by 86%, and in Georgia by 87%.

Teenagers establishing families is particularly troubling because of the high probability that families headed by unwed mothers will spend some and perhaps most of their lives in poverty. In fact, there is increasing evidence that having children at a young age is a major barrier to ever escaping the cycle of poverty that seems to be both a cause and consequence of teenage pregnancy. Statistically, <50% of teen mothers finish high school and only about 2% complete college.[3] People do not choose to be poor. Their choices, however, can keep them so.

Early childbearing has a number of other significant negative consequences. The offspring of teenagers are at increased risk for numerous health problems, including birth defects, low weight at birth, a high infant death rate, and many serious intrapartum problems. These children are also at substantial risk for lower intellectual and academic achievement, social behavior problems, and becoming teenage parents themselves. In essence, these young mothers who are unskilled, unschooled, and inexperienced are creating a very large number of our new families.

The significance of new families from teen birth is a particularly severe problem in our states.[3] In 1988 South Carolina was ninth in the country with new families begun with a teenage mother, Georgia was twelfth, North Carolina fourteenth, Florida twenty-second, and Virginia twenty-eighth. These new families contribute tremendously to the dollars we spend for teenage childbearing each year for aid to families with dependent children, food stamps, and Medicaid. In the last year for which figures are available,[4] the fiscal year 1986 through 1987, these amounts were $378 million dollars in Florida, $355 million in Georgia, $332 million in North Carolina, $198 million in Virginia, and $132 million in South Carolina. Specifically, in Virginia we have about 55 teenage pregnancies per day with 80% of them to unwed mothers.[5]

Virginia spent $198 million dollars in 1987 to support families that resulted from adolescent pregnancies and $126 million in 1988 to support the 8150 first birth babies born to teen mothers, or an average of $15,500 per baby. If we could have prevented 1% of the pregnancies that resulted in first birth babies that year, we could have saved $1,270,000 in 1988 and approximately $2 million in 1989. Also, just imagine what impact we could have with just 10% of the above amounts if it were used for prevention. Keep these figures in mind when you speak to your elected officials.

Specifically, there are several areas where your support and influence are most important. To have a significant impact, preventive programs must begin with very young children at a stage where the likelihood of developing strong socialization, communication, decision making, goal setting, and social resistant skills is the keenest. In this regard, one of our most important preventive programs is family life education, and we can be proud that our states all have a mandated policy for this in our public schools. In Virginia, Georgia, and North Carolina this begins in kindergarten and continues through at least the eighth grade.[6] Unfortunately, in Florida and South Carolina it is only mandated in the sixth, seventh, and eighth grades. I would encourage those of you from those two states to work to get this moved back to start in kindergarten.

When you consider the influence of the popular media on children's attitudes, the age of the onset of puberty, and the surmounting peer pressure to exploit sexual feelings associated with puberty, you will understand the importance of starting this program in kindergarten. Another important reason to begin early is the fact that the age of sexual initiation appears to be decreasing, as evidenced by the growing number of pregnancies in girls under the age of 15 and the estimate that in 1988 78% of adolescent girls and 86% of adolescent boys were sexually active by the age of 20. A more recent survey by the Centers for Disease Control showed that 40% of ninth graders were sexually active, and by the twelfth grade this figure was up to 72%.

Saying "no" and postponing sexual intercourse until a person is emotionally and psychologically ready is a value that should be the foundation of any family life education program. However, we must emphasize the well known fact that such a program has no impact on the behavior and attitude of students already sexually active at the start of the program. Therefore another essential part of family life education is information about contraception. Such information is inclusive in the programs in Virginia, Florida, and Georgia, but in North and South Carolina its inclusion is under local control of each community, which means great inconsistency as to its effectiveness.

In answering critics of family life education, we can assure them that the main purposes and objectives of any family life education programs are the promotion of positive health practices, critical thinking, learning to say "no," and avoiding risk-taking behavior and building skills in communication, responsible decision making, and assertiveness.

Schools cannot and should not replace the role played by family and religion in values education, but they can reinforce positive attitudes and behavior and counteract negative influences on the students.

Medical care to our teenagers provided by our schools and provided by us as health care providers can have a great impact on teenage pregnancy. Many teenagers, particularly the poor and minorities, have few resources to address their health needs because of inadequate health insurance, poor health care utilization, and a fragmented delivery system.[7] In the ideal situation comprehensive health care programs could respond with the health information and preventive care needs unmet by society. Such programs would have a high utilization rate because they are confidential, user friendly, and convenient.

Unfortunately, the status of school health care services in our states seems as fragile as the health of those they are designed to serve. We all have inadequate funding, inadequate school health personnel, and few opportunities for primary preventive care and health promotion activities. To be effective this is what we should strive for our school programs to be. First, there should be a comprehensive state health program that sets minimum standards for practice in all school districts. Second, adequate health care personnel with orientation, training, and health-related sources should be available in all the schools. Third, a school health advisory board for each community should monitor and evaluate the effectiveness of such programs. Finally, a commitment from the medical communities is needed to support and nurture these programs.

In our own specialty improved cooperation and communication with pediatricians and family physicians would improve the effectiveness of health care for adolescents, and of course, because of earlier sexual activity, the need is more urgent than it has ever been. One survey of college freshman showed that 78% had never received any counseling on sexually-transmitted diseases from their primary care physician and 70% had received no counseling on contraception.[8] As primary care providers and specialists, we should consider education regarding sexuality and sexually-transmitted diseases and counseling about contraception an essential part of our care for all teenagers. Adolescents should continually be queried about sexual activity and if they are sexually active, their sexual practices, feelings about sex, understanding of, and concern about the risk of pregnancies and sexually transmitted diseases, including acquired immune deficiency syndrome, should be discussed. In this setting confidentiality, empathy, and a nonjudgmental attitude are extremely important. Isn't it tragic when one of our patients needs treatment for a sexually transmitted disease or unplanned pregnancy because we did not take the time to present this information?

Our most urgent need in teen pregnancy prevention is greater availability of contraceptive services for adolescents, because accessible contraceptive availability is the only strategy documented to show a direct impact on teenage pregnancies. The real problem is that it is more comfortable for communities and parents to focus on abstinence rather than to work with sexually active adolescents to increase their access to contraceptive care. It is very clear from the literature that providing contraceptives will prevent pregnancy and not promote or encourage sexual promiscuity and that withholding contraception will not prevent sexual activity. These facts have been known for some time, which makes it hard to believe that the entire United States family planning program is funded only at about $130 million annually when the government could triple this investment for the cost of a single Stealth bomber.[9]

We must also work to overcome the numerous reasons teenagers do not use contraception. The most important is the lack of motivation to prevent an unplanned pregnancy, but other reasons, including real and perceived barriers to health care, fear of specific methods, hesitancy to reveal sexual activity, and the sense of invulnerability that teenagers have.

Teen pregnancy prevention is a very complex problem that requires overcoming a number of roadblocks if we are going to be successful. We must respond with the facts to the belief by some that adolescent pregnancy is an issue that affects minorities and the poor and does not merit policy makers' attention and by responding to the belief that adolescent sexuality should be addressed only in the home. We must also overcome the very organized but small vocal group of parents and

community members who oppose comprehensive family life education, school-based health centers, and the provision of contraceptives to teenagers. To do this we must rapidly widen advocacy efforts and community support.

Unfortunately, adolescent pregnancy prevention has not been a "frontburner" issue for state policy and budget makers, so we must constantly remind them to pay now for the prevention of at risk behavior or pay later as we do now with an ill-equipped work force, crowded prisons, and increased expenses for welfare, Medicaid, drug rehabilitation, and treatment and law enforcement programs.

In conclusion, I hope this address will challenge each of you to become more vocal and perhaps outraged about the lack of attention to prevention efforts by our federal, state, and local governments. We must join with these bodies to give the problem of adolescent pregnancy visibility and make its prevention a priority. Individually, we must offer contraceptive advice and services as part of our care for adolescents and encourage and educate our pediatric and family practice colleagues to do the same. Collectively, in each state we must support a statewide council on teen pregnancy prevention and support an extensive network of individuals and organizations working together at all levels of government. We must encourage innovative reforms in the welfare system, such as was recently passed in New Jersey where they voted to reduce or eliminate welfare benefits to able bodied recipients whose children are over 2 and who refuse to work or go to school and to deny additional benefits other than medical care to women already on welfare who have additional children. To be effective, a coordinated system must be developed to meet the health, social, and educational needs of teenagers, both male and female, and must include increasing the life options available to them and increasing their capacity to make decisions, particularly in the area of sexuality. Even in this recession we must seek these services and resources and convince our legislatures that prevention is much cheaper in the long run than the treatment of the consequences of inadequate programs.

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Attenuated proestrous luteinizing hormone surges in middle-aged rats are associated with decreased pituitary luteinizing hormone-β messenger ribonucleic acid expression

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OBJECTIVE: This study determined whether attenuated preovulatory luteinizing hormone surges in aging rats are associated with a decrease in pituitary luteinizing hormone content or luteinizing hormone β-messenger ribonucleic acid expression on proestrus.
STUDY DESIGN: Blood samples were taken every 90 minutes from 1:30 to 10:30 PM on proestrus in young (n = 8) and middle-aged (n = 12), regularly cyclic rats for plasma luteinizing hormone determination. On the next proestrus at 12 noon, rats were killed and the pituitaries were removed for luteinizing hormone content determination by radioimmunoassay and luteinizing hormone β-messenger ribonucleic acid expression by dot blot analysis. Results were analyzed by one-way analysis of variance.
RESULTS: Seven of the middle-aged rats had attenuated luteinizing hormone surges while the remaining five females had surges similar to those of young rats. On the next proestrus, all rats had similar quantities of pituitary luteinizing hormone. However, luteinizing hormone β-messenger ribonucleic acid expression in middle-aged rats with attenuated luteinizing hormone surges was lower ($p < 0.05$) than that of middle-aged and young rats with normal surges.
CONCLUSION: Decreased luteinizing hormone β-messenger ribonucleic acid expression, but not pituitary luteinizing hormone content at 12 noon on proestrus is correlated with attenuated luteinizing hormone surges in middle-aged rats. (AM J OBSTET GYNECOL 1992;167:303-7.)

Key words: Luteinizing hormone, luteinizing hormone β-messenger ribonucleic acid expression, female reproductive aging, pituitary, ovulation

Aging in the female rat is characterized by a progressive loss of regular estrous cyclicity and reproductive function.[1-4] During middle age (10 to 12 months), the estrous cycle patterns of female rats change from regular 4-day cycles to irregular, extended cycles (≥6 days).[5] These irregular cycles are characterized by extended periods of vaginal cornification reflecting sustained estrogen release.[5] After a transitional period of irregular cyclicity, female rats display sustained cornified vaginal epithelium and enter the anovulatory condition described as persistent estrus.

Although the neuroendocrine mechanisms associated with the age-related loss in regular ovulatory cyclicity are currently unknown, previous studies have revealed that the proestrous luteinizing hormone (LH) surges are attenuated in some middle-aged rats.[6-8] Furthermore, female rats with attenuated proestrous LH surges cease to display regular estrous cyclicity within 2 months, whereas rats of similar age with normal LH surges continue to cycle regularly beyond 2 months.[9] Thus changes in neuroendocrine function can be identified in aging rats before the loss of regular estrous cyclicity.

The following experiments were conducted to investigate further the possible alterations in the hypothalamic-pituitary axis of the aging female rat, alterations that are associated with the loss of regular estrous cyclicity and ovulatory function. To determine if attenuated LH surges are related to decreases in pituitary LH synthesis, these studies examined the relation between the magnitude of the proestrous LH surge and pituitary LH content and LH-β gene expression in young and middle-aged rats.

Material and methods

Animals. Young (4 months) virgin and middle-aged (10 to 12 months) retired breeder female Long-Evan rats (Charles River Lab, Wilmington, Mass.) were maintained under standard laboratory conditions at room temperature (23° to 25° C) with a lighting schedule of

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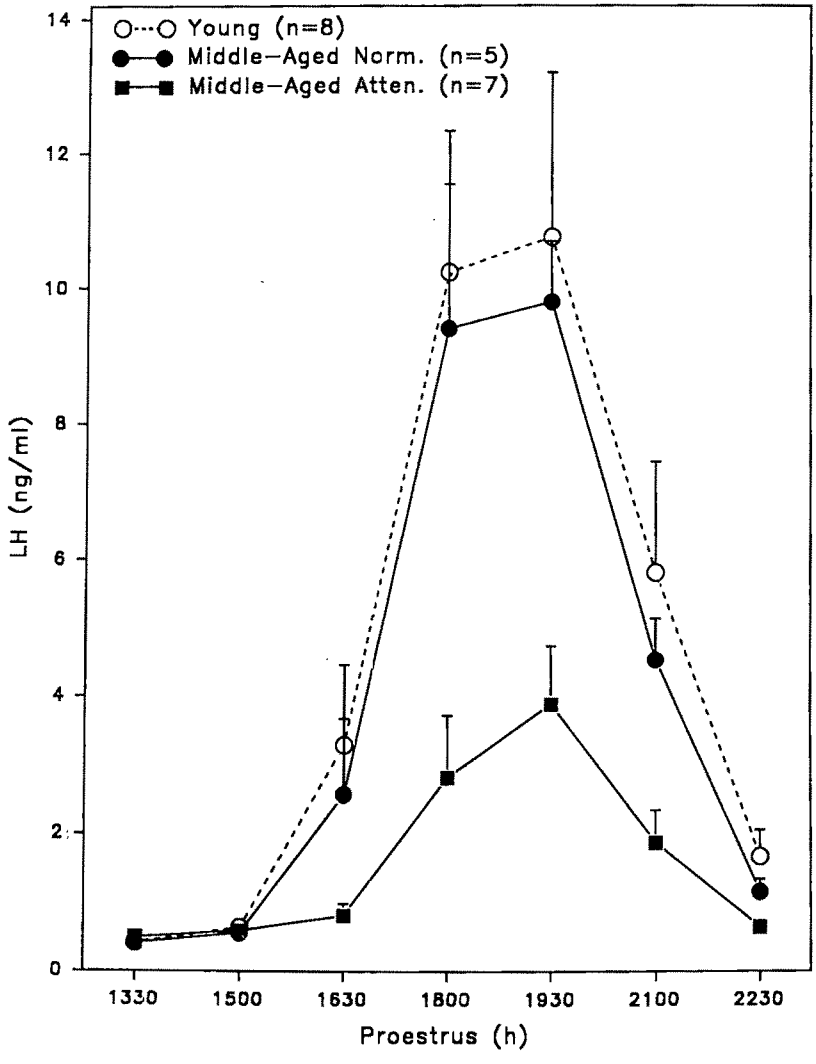


Fig. 1. Patterns of LH secretion in young *(open circles)* and middle-aged female rats from 1:30 PM *(1330 hours)* to 10:30 PM *(2230 hours)* on proestrus. Middle-aged females demonstrating peak LH values of >7 ng/ml were considered to have normal LH surges *(solid circles)*, whereas those middle-aged rats with peak LH values of <7 ng/ml were designated attenuated *(solid squares)*.

lights on from 5 AM to 7 PM daily. Food and water were available as desired. Daily vaginal smears were taken to monitor estrous cyclicity. Only animals demonstrating at least three consecutive 4-day estrous cycles were used for these studies. Guidelines for the care and use of these animals have been approved and followed in these experimental studies.

Experimental design. Groups of middle-aged (n = 12) and young (n = 8) regularly cycling rats were implanted with jugular vein catheters on diestrous day 2 between 10 AM and 1 PM according to the methods of Harms and Ojeda.[10] After this procedure, the rats were housed in individual cages, and on the next day, proestrus, blood samples were collected at 90-minute intervals from 1:30 to 10:30 PM for plasma LH determination by radioimmunoassay.

At 12 noon on the following proestrus, rats were killed by decapitation and the anterior pituitaries were rapidly removed, frozen, weighed, and stored at −80° C. At the time of assay, frozen pituitaries were thawed and sonicated in 300 μl of 0.01 mol/L phosphate-buffered saline solution (pH 7.5) for LH content determination and total ribonucleic acid (RNA) isolation. Before RNA extraction a small aliquot (20 μl) of homogenate was removed for determination of pituitary LH content. Total RNA was isolated by acid-guanidinium-thiocyanate-phenol-chloroform extraction followed by alcohol precipitation as previously described.[11]

Dot blot analysis. Total RNA (5 μg) from each pituitary was denatured and transferred to a Nytran nylon membrane through a 96-hole dot blot apparatus (Schleicher & Schuell, Keene, N.H.). To avoid differ-

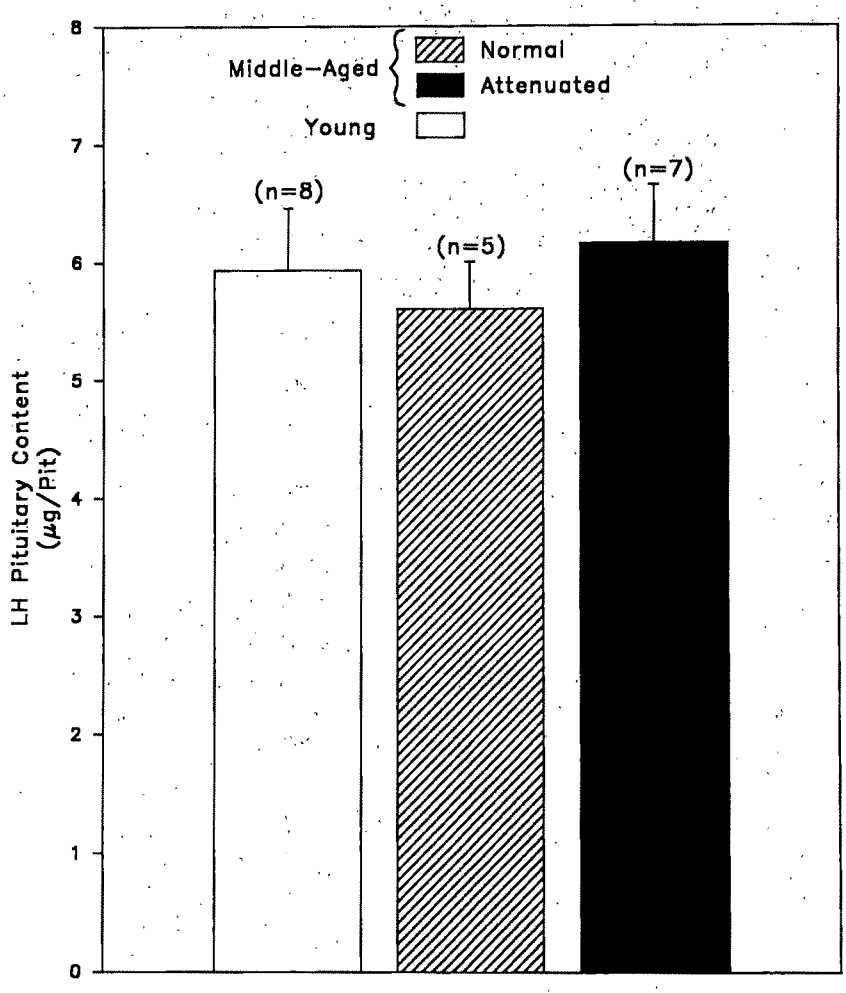


Fig. 2. Pituitary LH content at 12 noon in young *(open bar)* and middle-aged rats with previously normal *(striped bar)* or attenuated *(solid bar)* proestrous LH surges.

ences between dot blots, all samples from young and middle-aged rats were immobilized on one dot blot. Dot blots of standard dilutions of pooled pituitary RNA were also performed to verify linearity within the ranges of dot blot absorbances. The RNA dot blot was sequentially hybridized with denatured phosphorus 32–labeled deoxycytidine–labeled rat LH-β (kindly provided by Dr. W.W. Chin, Harvard Medical School) and chicken β-actin (kindly provided by Z.E. Zehner, Medical College of Virginia). Verification of each complementary deoxyribonucleic acid probe was determined by Northern blot analysis. The densitometric absorbance level of the dot blot autoradiographs was quantitated by laser densitometry. The relative absorbance of each sample as defined by the integrated hybridized area was expressed relative to that sample's β-actin absorbance to control for variation in RNA loading and transfer.

Hormone and data analysis. Quantitation of rat LH in plasma and pituitary homogenates was conducted by radioimmunoassay with reagents provided by the National Hormone and Pituitary Program. All LH values are expressed relative to the reference standard National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases rat LH-RP-2. Intraassay and interassay coefficients of variation calculated from pools of plasma were 8% and 12%, respectively.

Comparisons in the levels of pituitary LH contents and messenger RNA (mRNA) expression were performed by one-way analysis of variance, followed by the Newman-Keuls post hoc test for multiple comparisons when appropriate. A value of $p < 0.05$ or less was considered statistically significant.

Results

Proestrous plasma LH profiles. Fig. 1 illustrates the patterns of the plasma LH levels in young and middle-aged rats on the afternoon and evening of proestrus. Young females had proestrous LH surges that reached a maximum of 10.75 ± 2.46 ng/ml (mean ± SEM) at 7:30 PM. In these studies middle-aged rats with a peak LH level that was <7 ng/ml were considered attenuated

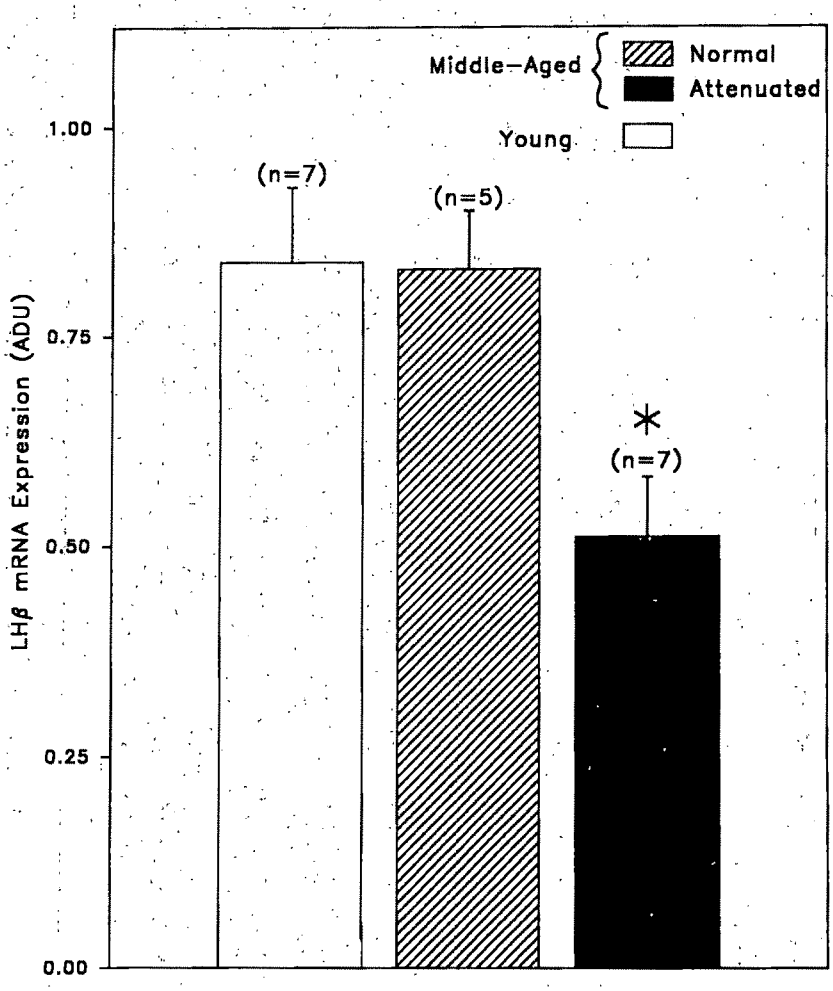


Fig. 3. Pituitary LH-β mRNA gene expression in young and middle-aged rats with previously normal or attenuated proestrous LH surges. Legend is same as for Fig. 2. *Asterisk*, $p < 0.05$ as compared with young and middle-aged normal rats.

whereas those with LH values >7 ng/ml were selected as normal. Five of 12 middle-aged rats had proestrous LH surges similar to those of young rats, reaching a maximum of 9.79 ± 0.90 ng/ml at 7:30 PM. The remaining seven middle-aged rats had markedly attenuated LH surges, which reached peak levels of only 3.87 ± 0.85 ng/ml at 7:30 PM.

Pituitary LH content. Fig. 2 illustrates the pattern of pituitary LH content in young and middle-aged rats on the following proestrus. The LH content of middle-aged rats with previously normal LH surges was 5.60 ± 0.41 μg per pituitary, similar to that of young rats (5.93 ± 0.53 μg per pituitary). Furthermore, the pituitary LH content of middle-aged rats with previously attenuated LH surges (6.17 ± 0.49 μg per pituitary) was similar to that found in both young and middle-aged rats with previously normal LH surges.

Pituitary LH-β mRNA expression. Fig. 3 illustrates the pattern of LH-β mRNA expression in young and middle-aged rats on the following proestrus. The relative mRNA expression of the LH-β subunit was similar in young rats (0.84 ± 0.09 arbitrary densitometric units) and middle-aged rats with previously normal LH surges (0.83 ± 0.07 arbitrary densitometric units). However, the relative expression of LH-β mRNA was markedly decreased ($p < 0.05$) in middle-aged rats with previously attenuated LH surges (0.51 ± 0.07 arbitrary densitometric units).

Comment

The present study characterized the relation in the magnitude of the proestrous LH surges in young and middle-aged rats to the pituitary LH content and LH β-subunit gene expression in the same animals at 12 noon on the subsequent proestrus. We hypothesized that the attenuated LH surges in middle-aged rats were related to decreased quantities of pituitary LH on proestrus. The results have demonstrated that the pituitary LH content is similar in young and middle-aged rats regardless of the magnitude of the LH surge. Thus it would appear that there are adequate quantities of LH for release in middle-aged rats with attenuated LH

surges. We further hypothesized that the attenuated surges were related to a decrease in pituitary LH synthesis. It is generally believed that the availability of the β-subunit is rate limiting for assembly and synthesis of this gonadotropin, whereas the common α-subunit is in excess.[12] This, in conjunction with the consensus that regulation of LH synthesis is at the transcriptional level,[12-14] indicates that measurement of LH-β mRNA expression may reveal differences in LH synthesis in middle-aged rats. Interestingly, the present study revealed that pituitary LH-β mRNA expression is decreased in middle-aged rats with attenuated LH surges before the onset of the surge. This may indicate several intriguing possible explanations that will require further investigation. The first possibility is that only the recently synthesized LH is available for release on proestrus. It has long been demonstrated that there are "releasable" and "nonreleasable pools" of pituitary LH.[15] Therefore, it is possible that in middle-aged rats with attenuated LH surges there is a limited amount of LH in the releasable pool, and this may be directly related to the quantities of newly synthesized LH β-subunits. Expression of LH-β mRNA is regulated by gonadotropin-releasing hormone (GnRH), which must be delivered to the pituitary at an appropriate frequency and amplitude for maximal expression.[16] These findings, combined with evidence that GnRH pulsatile frequency and amplitude are altered during aging,[17] may indicate that middle-aged rats with attenuated LH surges have an impairment in hypothalamic GnRH release. In addition to GnRH, estrogen also appears to play a major role in LH synthesis and LH-β mRNA expression.[18] Previous studies have shown that middle-aged rats with attenuated proestrous surges have elevated circulating levels of estradiol.[9] These elevated concentrations of estradiol could impair hypothalamic GnRH release and pituitary LH synthesis.

In summary, the study has revealed that pituitary LH-β mRNA expression is decreased in middle-aged rats with attenuated LH surges. This may indicate that alterations occur in ovarian steroid production, hypothalamic GnRH release, or pituitary LH synthetic and release mechanisms resulting in attenuated LH surges at middle-age and heralding the cessation of ovulatory function. These intriguing possibilities await further investigations.

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Outcome of corticotropin stimulation testing in women with androgen excess and ovulatory dysfunction

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OBJECTIVE: Our objective was to evaluate women with clinical signs or chemical evidence of androgen excess by corticotropin stimulation testing.

STUDY DESIGN: Seventy-six women with evidence of androgen excess were evaluated by corticotropin stimulation testing. Results were examined by plasma levels of dehydroepiandrosterone sulfate, androstenedione, and testosterone. Conception in those infertile women with androgen excess was also assessed. Data were evaluated with Fisher's exact test.

RESULTS: Of 41 women with dehydroepiandrosterone sulfate levels >2.8 μg/ml, 17 (41.5%) had a positive corticotropin stimulation test (stimulated 17α-hydroxyprogesterone value exceeded baseline value by 2.7 times). No statistically significant association was found between androstenedione or testosterone excess and a positive corticotropin stimulation test. In 14 infertile women with dehydroepiandrosterone sulfate levels >2.8 μg/ml and a positive corticotropin stimulation test, 7 (50%) conceived when given low-dose prednisone ($p < 0.005$).

CONCLUSION: Corticotropin stimulation testing is warranted in women with clinical signs of androgen excess and dehydroepiandrosterone sulfate levels >2.8 μg/ml. (Am J Obstet Gynecol 1992;167:308-12.)

Key words: Corticotropin stimulation testing

Congenital adrenal hyperplasia was described in 1865 by an Italian patholoist, de Crecchio; in the 1930s the measurement of adrenal metabolites in urine of newborn infants with genital ambiguity suggested that the condition resulted from a biosynthetic steroid defect.[1] With the development of radioimmunoassay procedures for the identification of specific steroids in the biosynthesis of cortisol permitted a more definitive diagnosis of congenital adrenal hyperplasia.[2]

In 1957 Decort et al.[3] reported patients who were seen during the peripubertal years with chronic anovulation and signs of androgen excess who had biochemical abnormalities similar to but less severe than those in congenital adrenal hyperplasia.[3] This condition was subsequently termed late-onset adrenal hyperplasia.[4] It is difficult to diagnose the late-onset form of adrenal hyperplasia because it can mimic polycystic ovarian disease.[5] However, it is important to differentiate late-onset adrenal hyperplasia from polycystic ovarian disease because appropriate treatment depends on the diagnosis of the underlying disorder.

In this study we report the results of corticotropin (ACTH) stimulation testing in 76 women with clinical signs of androgen excess to identify those women with late-onset adrenal hyperplasia and to treat them with low-dose prednisone.

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Material and methods

From April 1, 1990, through March 31, 1991, ACTH stimulation testing was performed in 76 women for evaluation of infertility or androgen excess. Of the women tested, 48 were infertile due to ovulatory dysfunction only, 18 were infertile due to multiple factors, and 10 were evaluated for acne and hirsutism. The latter group of women was not interested in pregnancy at the time of evaluation. All women were evaluated initially by measurement of plasma follicle-stimulating hormone, luteinizing hormone, prolactin, androstenedione, testosterone, and dehydroepiandrosterone sulfate (DHEAS). ACTH stimulation tests were performed primarily in women who had DHEAS levels >2.8 μg/ml ($n = 41$), in women with androstenedione levels >280 ng/dl or testosterone levels ≥0.8 ng/ml ($n = 24$), and in women with unexplained clinical signs of androgen excess ($n = 11$).

Blood samples were obtained by venipuncture at random in women who had a history of chronic anovulation and on any day between cycle day 7 and 11 in women who gave a history suggesting ovulation. Hormones were measured by radioimmunoassay by SmithKline Beecham Clinical Laboratories of Atlanta, Georgia.

ACTH stimulation testing was performed at random

Table I. Concentrations of androgens

Variable	*No.*	*Mean ± SD*	*Range*
DHEAS (μg/ml)	76	2.83 ± 1.03	1.0-5.9
Androstenedione (ng/dl)	76	281.55 ± 137.82	100-900
Testosterone	76	0.53 ± 0.37	0.2-2.6
17α-Hydroxyprogesterone ratio*	76	2.44 ± 0.89	0.9-5.3

*Ratio of post-ACTH to pre-ACTH administration.

Table II. Androgen levels by ACTH stimulation test outcome

Androgen	*No.*	*Positive test result*		*p Value**
		No.	*%*	
DHEAS				
≤2.8 μg/ml	35	10	28.6	0.24
>2.8 μg/ml	41	17	41.5	
Androstenedione excess†				
No	61	24	39.3	0.16
Yes	15	3	20.0	
Testosterone				
≤0.8 ng/ml	73	26	35.1	0.27
>0.8 ng/ml	3	2	66.7	

*Fisher's exact test.

†Androstenedione >280 ng/dl DHEAS <2.8 μg/ml.

in anovulatory women and on any day between cycle day 7 and 11 in women who gave a history suggesting ovulation. ACTH stimulation testing was performed as follows: Subjects were given 1 mg dexamethasone orally at 10 PM the night before the ACTH stimulation test. Dexamethasone was administered to suppress adrenal glucocorticoid secretion. The following morning at 8 AM, a scalp vein needle was inserted in the forearm of each subject. A baseline sample of 7 ml blood was obtained. Immediately thereafter, 250 μg ACTH (Cortrosyn, Organon, W. Orange, N.J.) was injected over 30 seconds. A heparin flush was placed in the intravenous line. One hour later, a second sample of 7 ml of blood was obtained.

The serum of the two blood samples was separated from the red blood cells and assayed by radioimmunoassay for 17α-hydroxyprogesterone. Results were expressed in nanograms per deciliter. An ACTH stimulation test result was considered to be positive if the stimulated (1-hour sample) was 2.7 times greater than the baseline sample.

Infertile women with positive ACTH stimulation tests were placed on 2.5 mg prednisone daily at bedtime. Those women who did not wish to conceive were placed on oral contraceptives along with prednisone.

Data were evaluated with Fisher's exact test. Differences were considered to be statistically significant if $p < 0.05$.

Results

The mean concentrations of androgens measured and the mean ratios of ACTH stimulation tests are presented in Table I. Associations of androgen studies with ACTH stimulation test outcome are presented in Table II. No statistically significant differences were found between the proportions of women with positive ACTH stimulation tests and the parameters set for normal and abnormal androgen values. However, 41% of women with DHEAS >2.8 μg/ml had a positive ACTH stimulation test compared with only 29% of those with DHEAS ≤2.8 μg/ml.

A statistically significant association was found between ACTH stimulation test results and subsequent pregnancy in women who received low-dose prednisone. Ten of 25 (40%) women with a positive ACTH stimulation test conceived within 6 months of the initiation of 2.5 mg prednisone daily (Table III).

Reasons for ACTH testing by test result and pregnancy are presented in Table IV. In the 14 women with DHEAS levels >2.8 μg/ml who subsequently had a positive ACTH stimulation test, seven (50%) concevied compared with only 1 of 19 (5.3%) who had a negative test. All conceptions occurred within 6 months of the initiation of low-dose prednisone therapy.

Comment

The most likely causes of acne, hirsutism, and irregular menstrual cycles are polycystic ovarian disease and late-onset adrenal hyperplasia. The differential diagnosis can be approximated by history, physical examination, and measurement of plasma gonadotropins and androgens, but definitive diagnosis of late-onset adrenal hyperplasia requires demonstration of excess of precursor hormone and deficiency of product hormone

Table III. ACTH stimulation test outcome by pregnancy

ACTH outcome	*No.*	*Pregnancies*		*p Value**
		No.	*%*	
Negative	39	6	15.4	0.04
Positive	25	10	40.0	

*Fisher's exact test.

Table IV. Reasons for ACTH testing by ACTH test result and pregnancy

Reason	*No.*	*Pregnancies*		*p Value**
		No.	*%*	
DHEA >2.8 μg/ml				
Negative test result	19	1	5.3	0.005
Positive test result	14	7	50.0	
Androstenedione >280 ng/dl				
Negative test result	10	3	30.0	1.00
Positive test result	2	1	50.0	
Unexplained excess				
Negative test result	10	2	20.0	.00
Positive test result	9	2	22.2	

*Fisher's exact test.

at the point of enzymatic abnormality. DHEAS is a steroid conjugate that is primarily the product of adrenal secretion. In conditions of adrenal enzymatic deficiency that cause late-onset adrenal hyperplasia, DHEAS is usually elevated. However, this hormone is not always found in excess in men and women with this condition; additional screening is required to establish a diagnosis of late-onset adrenal hyperplasia.[6]

ACTH stimulation testing is considreed to be the diagnostic test of choice to establish the diagnosis of late-onset adrenal hyperplasia. However, not all women with acne, hirsutism, or ovulatory dysfunction should undergo testing. The plasma concentration of DHEAS or 17α-hydroxyprogesterone is used as the discriminating test to indicate performance of an ACTH stimulation test.[7]

In recent years there has been much debate about the value of ACTH stimulation testing.[8, 9] Moreover, there are several methods for peforming this procedure.[10] In this study we set the lower limit of follicular phase plasma DHEAS concentration at 2.8 μg/ml for subsequent ACTH stimulation testing. This cutoff value was not a good discriminator because 10 women (29%) with DHEAS levels ≥2.8 μg/ml had a positive ACTH stimulation test. If we had chosen a higher level, e.g., 4.0 μg/ml, the predictive value of DHEAS would have been greater; however, several infertile women in this study (who subsequently conceived) would have carried the diagnosis of polycystic ovarian disease and would not have received low-dose prednisone therapy. Perhaps these women would have conceived without prednisone, yet it is tempting to conclude that their prompt conceptions were related to the diagnosis and treatment of late-onset adrenal hyperplasia.

When we performed ACTH stimulation tests in women with unexplained clinical signs of androgen excess or in women with DHEAS levels <2.8 μg/ml but with androstenedione >280 ng/dl or testosterone ≥0.8 ng/ml, we were unable to find an association with a greater number of positive ACTH stimulation tests. Only five women with an elevated androstenedione or testosterone but a DHEAS <2.8 μg/ml had a positive ACTH stimulation test. None of 11 women with unexplained androgen excess had a positive ACTH stimulation test.

From this study we conclude that women with clinical signs of androgen excess who have a midfollicular phase DHEAS level >2.8 μg/ml should undergo subsequent ACTH stimulation testing. This level of discrimination will identify women with mild, late-onset adrenal hyperplasia who may otherwise go undetected. If pregnancy is the desired outcome, administration of low-dose glucocorticoid suppression therapy may facilitate conception by normalizing the female reproductive cycle.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

Dr. Richard L. Wing, Charlotte, North Carolina. The author has studied a group of patients with either clinical or biochemical evidence of androgen excess. On the basis of a discriminatory DHEAS level <2.8 μg/ml, an ACTH stimulation test was performed. A positive ACTH stimulation test in this study identified women with mild, late-onset adrenal hyperplasia. With low-dose prednisone therapy a statistically significant number of these patients conceived compared with those whose stimulation test was negative. The author's comments suggest that ACTH stimulation testing in an androgenic group of patients would differentiate between late-onset adrenal hyperplasia and polycystic ovarian disease.

Gynecologists who treat infertility patients must answer this question: should an ACTH stimulation test be performed on the appropriate patient, and which one is appropriate? If late-onset adrenal hyperplasia is diagnosed, the treatment with low-cost, low-dose glucocorticoids could be used to help these patients conceive before proceeding to more expensive treatment protocols.

Measurements of testosterone, androstenedione, DHEAS, and 17α-hydroxyprogesterone have been shown to overlap in late-onset adrenal hyperplasia and in patients with other causes of hirsutism.[1] In 1982 Ayers[2] published a report on 38 patients who were separated into high and low DHEAS groups. After ACTH stimulation, the mean 17α-hydroxyprogesterone values in the high DHEAS group only doubled compared with the basal level. His study also concluded on the basis of DHEAS levels that two groups of patients with polycystic ovarian disease existed. The low DHEAS group represented the classic Stein-Leventhal polycystic ovarian disease type I patient. The elevated DHEAS group represented the polycystic ovarian disease type II patient whose adrenal steroidogenesis was thought to be under tonic hyperstimulation to maintain normal cortisol production. Adrenal suppression was considered to be the more physiologic therapy for the latter group.

Accurate diagnosis of late-onset adrenal hyperplasia has been obtained by the measurement of 17α-hydroxyprogesterone after ACTH stimulation.[3, 4] In this presentation the authors state that an ACTH stimulation test result was considered positive if the stimulated sample was 2.7 times greater than the baseline sample. Where are the data to suggest that 2.7 times the baseline 17α-hydroxyprogesterone value is a positive test for ACTH stimulation? Would the basal levels obtained in this study group serve as a discriminating test for ACTH stimulation? On the basis of the nomogram produced by New et al.,[3] how many of the patients in this study would be considered to have positive results by those criteria?

Glucocorticoids have been beneficial in the treatment of infertile patients with hirsutism and with polycystic ovarian disease.[5, 6] Steinberger et al.[7] reported a 44% pregnancy rate in infertile women treated with 7.5 mg prednisone. Zarate et al.[8] reported a 36% pregnancy rate. Rodriguez-Rigau et al.[9] used 5 to 10 mg prednisone daily and induced ovulation in five of 14 amenorrheic hirsute women and 10 of 11 anovulatory patients. Prednisone therapy in a dosage of 2.5 to 10.0 mg/day was used by Farris et al.[10] to treat patients with late-onset adrenal hyperplasia diagnosed only on an elevated 17-ketosteroid level >15 mg/24 hours. All 13 patients with infertility conceived. How many of the prednisone-treated patients were ovulating before treatment? How many of the nontreated patients were ovulating? This information would be helpful in analyzing this study's findings. It would also be of great interest to give prednisone therapy to all of the patients in the author's study group and to determine their pregnancy rate.

Other interesting observations would be to measure the level of suppression of 17α-hydroxyprogesterone, DHEAS, testosterone, and cortisol with a bedtime 2.5 mg prednisone dose.

The author has, in this study, found an association between ACTH stimulation test results and subsequent pregnancy in women who received low-dose prednisone. Future studies may benefit by establishing a control group with positive ACTH stimulation testing that is not treated with prednisone. Likewise, the negative ACTH stimulation group might also be divided into a treated and nontreated group.

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Dr. Bates (Closing). I think that Dr. Wing has come to the nub of the issue in making the diagnosis of late-onset adrenal hyperplasia. That is how you do it and how you differentiate this disorder from polycystic ovarian disease. The debate continues to rage and I do not think it will be settled this morning.

There is a lot of overlap between polycystic ovarian disease and late-onset adrenal hyperplasia. We know that the ovary produces a small amount of DHEAS and can also produce elevated levels of androstenedione. It can be very confusing as to the source of these precursor hormones in androgen excess disorders. The way we attempt to make a diagnosis is to collectively stimulate the adrenal glands. There is no uniformity in the literature as to how this test is best performed. Azziz and Zacur[1] in 1989 performed intravenous ACTH stimulation testing over 1 hour. They set the limits for positive tests so stringently that only 2% of their patients had a positive ACTH test. On the other hand, Seigel et al.[2] reported in 1990 a positive ACTH stimulation test at a change from 100 to 300 ng/ml in the concentration of 17α-hydroxyprogesterone. They tested by doubling the amount of ACTH infused by bolus administration and obtained blood samples 30 minutes later. Therefore one of the difficulties we have is standardizing of the testing procedure and evaluating the test outcome.

We selected a value 2.7 times because in the few normal patients in whom we have done this test the ratio of 17α-hydroxyprogesterone, stimulated as compared with suppressed, is approximately 2, and two standard deviations from this mean falls at about 2.7 times. Because we are testing at 1-hour intervals we will not produce as high a stimulated 17α-hydroxyprogesterone value as would a test peformed at 30-minute intervals.

It is also well known that the pregnancy rate is variable if you administer low-dose glucocorticoid therapy to women with polycystic ovarian disease. Perhaps the mechanism for this is to suppress the overall androgen milieu and to allow normal ovulatory function. Forty-one women in our study were ovulating. These women have been infertile for a long time and many have luteal phase defect, as diagnosed by low progesterone levels and shortening of the luteal phase of their menstrual cycle.

It would be nice to have a control group in this study. It is very difficult, though, in the private practice of infertility to establish a control group and alter their therapy. These are women who have been infertile for a long time and they have experienced the failure of not conceiving over a long period of time, but I do agree that it would be nice to have a randomized study and have a placebo versus a low-dose progesterone group.

Finally, congenital adrenal hyperplasia is probably the most widely studied metabolic disease. It is well known that the genetic disorder is carried on the short arm of chromosome six and the localization of this gene has been identified. Some authors have performed a number of studies and have isolated the gene for 21-hydrolase activity. It is known that a shift in the base paranucleotides from A to C or A to G will produce this disorder. Moreover, a shift in the exon isolating this gene from others will also produce late-onset adrenal hyperplasia. Approximately 1% of people in the United States carry this deficient gene. Approximately 5% of Ashkenazi Jews in eastern Europe carry this genetic disorder. Therefore it is a very common disorder. A specific diagnosis can be made by deoxyribonucleic acid hybridization but that is as yet not available in clinical practice.

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Etiologic factors of recurrent abortion and subsequent reproductive performance of couples: Have we made any progress in the past 10 years?

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OBJECTIVES: We hypothesize that the diagnostic yield and pregnancy outcomes of patients with recurrent abortion have improved over the past 10 years.
STUDY DESIGN: The study was performed in an academic medical center. Diagnoses and outcomes for group A, a published series of 100 patients investigated for recurrent abortion in the section between 1968 and 1977, was compared with those for group B, the 131 patients seen between 1987 and 1991. A standardized protocol was followed, enhanced by new techniques and autoimmune investigations in the latter group. Results were compiled retrospectively. Descriptive statistics and χ^2 analysis were used.
RESULTS: No cause could be found in 37% of patients in group A compared with 24% of couples in group B ($p < 0.05$). No clear difference could be shown in the subsequent outcomes of pregnancies.
CONCLUSIONS: Our ability to establish a cause of recurrent abortion has improved slightly over the past 15 years. The gain is not yet reflected in successful pregnancy rates. Multicenter trials are indicated. (Am J Obstet Gynecol 1992;167:313-21.)

Key words: Recurrent abortion, pregnancy outcome, autoimmune disease

Diagnosis and management in the couple with recurrent abortion are still among the most difficult areas in reproductive medicine.[1] Uterine anomalies and balanced chromosomal rearrangements in parents are the only two universally accepted etiologic factors in humans. Corpus luteum defect, associated ovulatory disorders, and incompetent cervix are considered by most as likely causes. Many changes have occurred in this field over the past 10 years. The technology available for the workup of patients has improved. Autoimmune disorders have also gained broad acceptance as etiologic factors. The role of infections, alloimmune disorders, elevated gonadotropins and androgens, on the other hand, are still under debate.[2]

We have previously reported on a series of 100 couples with recurrent abortion studied during the period from 1968 through 1977.[3] In 37 of these couples no specific etiologic factor could be established. The purpose of the current report is to establish the diagnostic yield of an updated diagnostic protocol applied to a group of 131 couples seen from 1987 through 1991. These results were compared with those of our previously published series to see whether we have improved in our ability to make a diagnosis in couples with recurrent abortion. The outcomes of pregnancies are also assessed.

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6/6/38056

Material and methods

All the couples included in this study were seen in the Reproductive Endocrinology Section at the Medical College of Georgia. Patients seen from 1968 through 1977 are referred to as group A and were described in a previous report.[3] Group B patients included all patients first seen for recurrent abortion between July 1, 1987, and June 30, 1991. In both groups couples were eligible if they had two or more documented abortions or one abortion associated with a phenotypically abnormal child. In group A documentation of the abortion was histologic whereas in group B it could be based on either histologic evidence or ultrasonography.

Patient population. All couples were referred to our section from gynecologists in Georgia and surrounding states because of a history of recurrent abortion or abortions with a malformed child. A small number of these couples were self-referred or were seen for prenatal genetic counseling, at which time the abortion history was discovered. Nearly all patients benefited from comprehensive health care insurance coverage.

During our first study period we were involved in an outreach genetic program. Most of the couples with a phenotypically abnormal child were enrolled through this pathway. This was not the case in the recent series. From 1987 to 1990 we were involved in a National Institutes of Health (NIH) multicenter study in the management of autoimmune pregnancy loss. Patients referred to our unit for inclusion into this clinical trial were excluded from the current study. Only three patients from the current study were randomized to one of the treatment protocols from the NIH trial.

All the patients were advised to avoid pregnancy and use mechanical contraception during the evaluation period. A full medical history, including a thorough genetic evaluation, was obtained, followed by a complete physical examination. Patients were routinely screened for systemic disease, including thyroid dysfunction. Anyone with a history of alcoholism and substance abuse was excluded from the study.

Cytogenetic studies. Karyotypes were obtained on all couples. Banding resolution has improved between our first and second series. In all cases a minimum of 20 cells were counted with at least two being karyotyped. Further studies, including ultra-high-banding resolution, were performed whenever a karyotype was under question. When a balanced translocation was found in either parent, the couple was counseled as to its prognosis and the necessity for prenatal diagnosis.[4, 5]

Structural studies of the uterus. All women were offered a hysterosalpingogram unless pregnant at the time. For group A patients, any intracavitary defect that was demonstrated on hysterosalpingography was further delineated through gynecography. Metroplasty was performed when appropriate. For group B patients, the initial study consisted of either a hysterosalpingogram or hysteroscopy. Pregnant patients underwent an ultrasonographic study in an attempt to identify any mullerian anomaly. Any abnormal hysterosalpingogram was followed by a hysteroscopy laparoscopy, as judged appropriate. Resection of uterine septa and intrauterine synechiae was performed through the hysteroscope. Incompetent cervical os was suspected on historic information and confirmed by vigilant cervical examination during pregnancy.

Endocrine function. Dating of all endometrial biopsy specimens was done by the authors with established criteria.[6] All patients with one abnormal biopsy specimen were strongly encouraged to have a repeat biopsy. Two out-of-phase biopsy specimens were required to make a definitive diagnosis of corpus luteum defect.[7] A single anovulatory biopsy specimen and a compatible clinical history were required to make the diagnosis of an ovulation disorder.

The endocrine function of patients in group A was determined by basal body temperature readings and timed endometrial biopsy. The biopsies were performed with rigid metal cannulas. Women with secretory biopsy specimens showing a discrepancy in endometrial development of ≥3 days were treated with preimplantation progesterone vaginal suppositories.[8] Those displaying markedly heterogeneous biopsy specimens or proliferative endometrium were treated by a combination of clomiphene citrate and progesterone vaginal suppositories. In group B the same approach was most often enhanced by determination of ovulation with home urinary luteinizing hormone (LH) testing, ultrasonographic follicular monitoring, and estradiol and progesterone assays. Endometrial biopsies were almost exclusively obtained with a flexible plastic cannula (Pipelle). Treatment protocols were individualized and consisted of progesterone vaginal suppositories, clomiphene citrate, dexamethasone, and human menopausal gonadotropin, in various combinations.[8]

Autoimmune studies. Autoimmune studies were not performed on any patient in group A. All of the patients in group B underwent evaluation for anticardiolipin antibodies (ACA), antiphosphatidylserine antibodies (PS), and lupus anticoagulant (LAC). Levels of immunoglobulins M and G were measured with a modified Loizou enzyme-linked immunosorbent assay with the control population consisting of normal first- and second-trimester pregnancy values.[9] All these tests were performed in the same laboratory coordinated through the NIH multicenter trial (Dr. Susan Cowchock, Thomas Jefferson University, Philadelphia). The results were converted to multiples of the median and interpreted as negative (<2), low (2 to 3), mid (3.1 to 4), or highly positive (>4). The LAC assay was performed according to standard technique, in our own laboratory, on any patient with an elevated activated partial thromboplastin time. Any test with an abnormal result was repeated at least once to confirm a true-positive finding. Mid or highly positive ACA or PS antibodies or a positive lupus anticoagulant test was considered as evidence for a probable autoimmune cause. Included in the same category were patients with an established diagnosis for systemic lupus erythematosus or other advanced autoimmune disease. Low ACA or PS antibodies were labeled as possible autoimmune etiologic factor.

All couples in this category were offered randomization in the NIH multicenter trial.[9] Those who declined were still offered one of the treatment protocols. Treatment protocols included no treatment, 80 mg daily of aspirin alone, 80 mg daily of aspirin with subcutaneous heparin every 12 hours, or 80 mg of aspirin and 20 mg of prednisone twice daily.

Alloimmune studies. No alloimmune study protocol was available during the 1968 to 1977 period. For

Table I. Identified etiologic factors in group B (n = 131)

	Total
Unknown	22 (17%)
Incomplete workup/no etiologic factor	19 (15%)
Probable	72 (55%)
Balanced translocation in one parent	3 (2%)
Müllerian anomaly	2 (2%)
Significant uterine anomaly	13 (10%)
Incompetent cervix	3 (2%)
Corpus luteum defect	31 (24%)
Severe ovulation disorder	14 (11%)
Mid or high positive levels of ACA or PS	21 (16%)
Positive lupus anticoagulant	0
Active systemic lupus erythematosus or other connective tissue disorder	3 (2%)
Possible	13 (9%)
inv 9 (p1q1) with family history of recurrent abortion	4 (3%)
Minor müllerian anomaly	8 (6%)
Minor uterine anomaly	0
Low positive levels of ACA or PS	9 (7%)
Other	5 (4%)
Hypothyroidism	11 (8%)
Hyperprolactinemia	10 (8%)
Diabetes	0
Minor ovulation disorder	14 (11%)
Infection	1 (1%)

Note: The same patient may have more than one diagnosis so that the subtotals exceed 131. The total equals 131.

patients in group B the available data about alloimmune etiologic factors in recurrent abortion was discussed.[10, 11] Couples were offered testing in the form of histocompatibility antigen typing followed by mixed lymphocyte cross-reactivity testing. Most couples declined this on financial grounds. Those individuals who underwent testing and were found to be positive were referred to outside centers for further evaluation and treatment but continued to undergo follow-up by our unit.

Other tests. The role of infection in recurrent abortion is highly debated.[12, 13] Patients in group A in whom no cause could be found were empirically treated with antibiotics. By contrast, patients in group B were evaluated through cervical cultures and a serum complement fixation assay for antichlamydial antibodies. Only those women with positive cultures or with high antibody titers (≥1:32) were treated.

During the 1987 through 1991 study period, several reports have appeared about the role of prolactin disorders, elevated LH, or elevated androgens in recurrent pregnancy loss.[14, 15] These were obtained liberally in patients presenting with suggestive histories and measured by radioimmunoassay. Women with elevated prolactin levels on the initial screen had a repeat study performed in a fasting state in the morning. If confirmed, computed tomographic or magnetic resonance imaging studies were obtained. If indicated, therapy was initiated with oral bromocriptine.

Management. Once all identifiable factors were corrected, patients were encouraged to achieve pregnancy. All couples were then allowed to conceive and to have postimplantation progesterone supplementation for up to 60 days of pregnancy. Other appropriate treatments were administered and monitored throughout the pregnancy.

Data analysis. Standard descriptive statistics were used for the analysis of our data accumulated between 1987 and 1991. Patients are counted only once and their further pregnancies are considered as outcome events only. Patients were grouped into five groups as listed in Table I. Confidence intervals were determined by the confidence interval for a population proportion within the same group and by the confidence interval for the difference between two population proportions formulas. A χ^2 analysis was also performed where appropriate.

Results

From 1968 through 1977 100 couples met the criteria for eligibility into the study. Thirteen of these couples were ascertained because of a history of one malformed child and at least one abortion. Because this particular subgroup was recruited through our genetic program, they will be excluded from the comparative study, leaving 87 couples from group A. During the 1987 through 1991 period, 131 couples were elevated for a history of two or more recurrent abortions.

Demographic data are not available for group A. The average age of women in group B was 30.0 years, with a range of 18 to 44. There were 108 white and 23 black women. The total number of pregnancies per patient ranged from two to eight. Eighty-seven women had no previous term pregnancy, 35 had one previous term

Table II. Frequency of probable etiologic factors for recurrent abortion in groups A and B

	Group A		*Group B (total No. of couples)*
	Total No. of couples	*No. without genetic history*	
Uterine anomaly	n = 100	n = 87	n = 114
No.	15 (15%)	15 (17%)	18 (16%)
95% Confidence interval	8%-22%	9%-25%	9%-23%
Balanced translocation	n = 100	n = 87	n = 131
No.	12 (12%)	12 (14%)	3 (2%)
95% Confidence interval	6%-18%	7%-21%	0%-4%
Other genetic	n = 100	NA	NA
No.	13 (15%)		
95% Confidence interval	6%-20%		
Endocrine	n = 100	n = 87	n = 117
No.	23 (23%)	23 (26%)	37 (32%)
95% Confidence interval	15%-31%	17%-35%	24%-40%
Autoimmune	NA	NA	n = 131
No.			24 (18%)
95% Confidence interval			11%-25%
Unknown	n = 100	n = 87	n = 112
No.	37 (37%)	37 (43%)	27 (24%)
95% Confidence interval	28%-46%	33%-53%	16%-32%

NA, Not available.

Note: 95% Confidence intervals were calculated within each group. See Table IV for comparison between groups.

delivery, and nine had two or more term deliveries. Seventy-five patients had a pure history of loss, including stillbirths and ectopic pregnancies. This constitutes a group referred to by many as primary aborters. Consecutive pregnancy losses occurred in 116 of the couples, although they were interspersed in 16. The most common occurrence was one term delivery followed by consecutive abortions. Thirty-six couples also had a history of infertility, with an ovulation disorder, endometriosis, and tubal factors being the predominant diagnosis. Six patients were already diagnosed with hypothyroidism, two with systemic lupus erythematosus, one with mixed connective tissue disease, and one with compensated renal failure of unknown cause. One patient was found to have carcinoma in situ of the breast during her workup. All other patients were in good health.

All patients in group A underwent a complete diagnostic evaluation. Fourteen patients in group B were pregnant at the time of their initial presentation. Another 27 did not undergo all of the core investigations. The most common reasons for an incomplete evaluation were a pregnancy occurring before the workup could be completed and the identification of an etiologic factor or of pathologic conditions, such as the detection of a balanced translocation, that prompted the patients to interrupt their attempts at pregnancy. Therefore 90 patients in group B underwent a complete workup.

Table I presents the results of the diagnostic investigations in series B. Patients may have more than one etiologic factor identified, and therefore the sum of categories may exceed the total. Table II compares the frequency of probable diagnoses between both series. The number of couples in group B who underwent each diagnostic maneuver is indicated.

The frequency of balanced parental translocations is shown in Table II. In addition, four patients in group B showed a 9(p1q1) pericentric inversion. This was not considered significant as an etiologic factor.[16]

Uterine anomalies in group B were classified as significant and incidental, depending on their type and severity. Table III shows this breakdown. Only significant uterine anomalies were listed in Table II.

In group B, 91 patients underwent at least one endometrial biopsy. In 60 patients, at least one biopsy was read as in phase. Ten patients refused a second biopsy after the first one had been read as out of phase. They were empirically treated with progesterone supplementation and are considered as having a corpus luteum defect. Twenty-one patients showed two out-of-phase endometrial biopsy specimens. Fourteen women in group B had a severe ovulation disorder and were included in the endocrine category. Fourteen other women displayed only subtle ovulatory disorders that were detected but thought to relate more to infertility than recurrent abortion. These were not included and are not listed in Table II.

Autoimmune tests were only available for women in group B. The women in all 131 couples underwent these tests. Twenty-one women had mid or high levels of ACA, PS antibodies, or a positive LAC screen on two occasions. Three women also had active connective tissue disorder of autoimmune origin, as listed above. All

Table III. Uterine anomalies identified in groups A and B

Anomaly	Group A (n = 100)	Group B	
		Significant	Incidental
Septum	10	2	0
Fibroids	1	5	3
Adhesions	0	8	4
Incompetent cervix	4	3	0

these cases were viewed as having a probable autoimmune cause and are listed in Table II. In addition, nine women consistently had low levels of ACA. For this study these low levels were considered as a possible cause for abortion, and these women are not included in Table II.[9]

Thirty-three couples had two or more probable causes of abortion. This excludes the association of corpus luteum defect and ovulation disorders. No specific association was more prevalent. One couple had balanced translocation and high positive autoantibodies.

No cause for recurrent abortion could be identified for 37 couples in group A. A comparable figure is more difficult to define for group B. There are 22 couples for whom no diagnosis could be made after a complete workup. In five other couples medical problems, such as hypothyroidism, were identified and corrected. Because there is much debate about the negative impact of such factors on pregnancy outcome, we have added these to the list of unknown diagnosis, for a total of 27 couples. There were 19 couples in whom no diagnosis could be made but where the workup was not completed in its entirety. We excluded these from our list of unknown causes, and the denominator for the unknown diagnosis is therefore 112 couples.

Thirteen couples had possible factors that are hotly contested in terms of their potential for recurrent abortion. They are not listed anywhere in Table II.

Additional data are not available for patients in group A. All but four women in group B were screened for thyroid disease. Hypothyroidism was newly diagnosed in five women, whereas two of the six women who were known to have this disorder were found to be undermedicated. Screening for chlamydia was performed in 96 women and yielded a single positive serologic result at 1:64. Prolactin levels were obtained in 113 women, and 10 women were found to have persistently elevated levels that were corrected by therapy with bromocriptine. Serum levels of testosterone, dehydroepiandrosterone sulfate, LH, and follicle-stimulating hormone were obtained in fewer than one third of patients, mostly in the context of ovulation disorder. These substances were found to be abnormal in about 10% of the patients tested. The numbers are too small to draw any inferences at present.

Table IV compares the diagnostic yield between groups A and B in two different ways. Because 13 patients in group A were ascertained through genetic referrals, these are excluded from the analysis. Therefore 87 couples are considered from group A. From group B, we used only those 90 couples who were not pregnant at presentation and who completed a workup. Both probable and possible etiologic factors are combined under "known diagnosis." First, we compared both of these groups in toto. On the basis of this analysis, we have not improved our efficiency at making a diagnosis in these patients ($p = 0.082$). This is also illustrated by the 95% confidence interval between the two groups.

Next, we repeated the analysis excluding chromosomal anomalies from both groups. This was done because our figure of 12% of chromosomal anomalies in group A is greater than in most series, where the prevalence ranges between 3% and 5%.[16] With these new parameters, we have improved our ability to make a diagnosis by close to 20% ($p = 0.018$) (95% confidence interval = 4% to 34%). This is a small difference, however, which holds true only if we combine probable and possible causes.

The outcome of subsequent pregnancies for patients in group A has been reported.[3] In summary, 80 couples elected to attempt pregnancy; an additional nine couples also attempted pregnancy but were in the multifactorial genetic factor group. Of the couples in whom a diagnosis was made and who attempted pregnancy, 34 out of 43 (79%) achieved a successful pregnancy. In those couples where no diagnosis could be made, 37 attempted pregnancy and 23 were successful (62%). In group B 35 couples were either lost to follow-up or elected not to pursue pregnancy. In the 22 couples where no diagnosis could be made, we have follow-up on 17. Eight (36%) of these eventually achieved a successful pregnancy, whereas five had only losses and four failed to achieve a pregnancy. In the 72 couples where a probable diagnosis was made, 24 were lost to follow-up or decided not to pursue a pregnancy. Of the remaining 48, 35 (73%) had live births, five had spontaneous abortions only, and eight were unable to achieve pregnancy (Table V). The minimum follow-up interval is 6 months and the maximum 4 years. These

Table IV. Compared diagnostic yield of a completed diagnostic workup

	1968-1977	*1987-1991*	*Difference (%) and 95% confidence interval*
Adjusted groups—All diagnoses			
Total No.	87	90	
Diagnosis	50 (57%)	64 (71%)	
			14 (0%-28%)
No diagnosis	37 (43%)	26 (29%)	
Adjusted groups—Excluding balanced translocations			
Total No.	75	87	
Diagnosis	38 (50.7%)	61 (70%)	
			19.3 (4%-34%)
No diagnosis	37 (49.3%)	26 (30%)	

For adjusted groups—All diagnoses, $\chi^2 = 3.020$ $p = 0.082$. For adjusted groups—excluding balanced translocations, $\chi^2 = 5.618$ $p = 0.018$.

numbers are too small to draw any conclusions in the outcomes between our various etiologic groups. No significant difference in outcomes is demonstrable between groups A and B.

Comment

The traditional workup of the patient with recurrent abortion has included structural evaluation of the uterine cavity, histologic assessment of the endometrium, parental chromosomal studies, and a thorough medical evaluation, looking for systemic disorders and thyroid dysfunction.[17] In the past 10 years several technologic changes have been recorded. Hysterosalpingography has been complemented by hysteroscopy. Endometrial sampling is easier because of the use of flexible sampling devices. Ovulation assessment is enhanced by urinary LH monitoring, ultrasonography, and hormonal assays. The banding resolution of chromosomal studies has improved. Screening for infections has improved through enhanced culture techniques and antibody assays.

In addition to the advent of this new technology, the area of immune pregnancy loss has come of age.[18, 19] Autoimmune factors, including the presence of ACA, PS, and LAC, have gained acceptance as etiologic factors in recurrent abortion. Alloimmune factors have been under scrutiny.[10, 11]

In this context it is important to assess if we have made any true progress in our management of couples with recurrent abortion. Various studies have looked at a similar breakdown of causes in this patient population.[1, 3, 20, 21] It is extremely difficult to compare series, however, because of varying definitions of what constitutes recurrent abortion, differing diagnostic protocols, and technical maneuvers.[2] The current study offers the opportunity of comparing the diagnostic yield of two standardized diagnostic protocols within the same institution at two different time periods. The protocol for the period of 1987 through 1991 is similar to that for the period of 1968 through 1977, except that it uses the latest available technology and includes testing for autoimmune disorders.

The breakdown of patients among the different categories, as outlined in Tables I and III, is consistent with other similar reports.[1, 20, 21] Chromosomal problems were seen less frequently in group B than in group A but are in effect closer to the majority of other surveys.[16, 22] Random clustering could account in part for this difference.[23] Another explanation is that referring physicians now order karyotype evaluations directly and manage these cases locally. The merits of uterine structural studies are confirmed by our data.

Evaluation of endocrine factors is now easier and more thorough with the advent of flexible sampling cannulas and urinary LH monitoring, coupled at times with ultrasonographic follicular monitoring. This remains a prevalent etiologic group and continues to gain broad acceptance.[8] The high prevalence of thyroid dysfunction and hyperprolactinemia supports routine screening for these disorders in this population.

Autoimmune antibodies were present in 16% of the total patient population. This figure is in line with previous reports from other sources.[19, 24] We feel it is well justified to obtain these in all patients with two or more abortions, although other reports contest this finding.[25] This may be linked to differences in laboratories. All of our assays were performed in the same laboratory, with high quality control. In parallel, our clinical experience with outside laboratories has been far less encouraging, and there is little correlation between different laboratories. These issues must be resolved before such tests become widely used clinically. In the meantime we would encourage interested clinicians to refer their samples to an established laboratory with a proved track record, specifically in the field of autoimmune pregnancy loss.

Table V. Outcomes of pregnancies by diagnostic categories in group B patients where a probable diagnosis was made

Diagnosis	*At least 1 live birth*	*Abortions only*	*No pregnancy achieved*
Balanced translocation (n = 1)	1	0	0
Uterine anomaly (n = 4)	3	0	1
Endocrine (n = 21)	14	1	6
Autoimmune (n = 8)	6	2	0
Translocation and autoimmune (n = 1)	1	0	0
Endocrine and uterine (n = 3)	3	0	0
Endocrine and autoimmune (n = 10)	7	2	1

We found little support for infection as an etiologic factor. This is in contrast with the findings of other investigators with comparable techniques.[12] It may reflect population differences or technical problems with the culture and assay systems.

The 33 couples who had more than one etiologic factor identified highlight the need for a complete workup in these patients. Stopping once a single cause is identified is ill-advised and may lead to inaccurate counseling and therapy. The couple with a balanced paternal translocation and high positive immunoglobulin G ACA titers in the wife is an excellent case in point.

When we tried to compare the results from the two series, several difficulties emerged. First was the ascertainment bias. Our referral base had evolved between the two study periods. We clearly saw fewer referrals for genetic indications. We must also wonder whether referring physicians now feel more comfortable ordering karyotypes in these couples and handling counseling issues when balanced translocations are identified. The same could be true of the diagnosis and management of müllerian anomalies. Second, we are still plagued by the fact that only two causes are clearly established for recurrent abortion: balanced parental translocations and severe uterine anomalies. Last, even with the accumulation of data over a relatively long period of time in a tertiary referral center, numbers remain small and it is difficult to draw definitive conclusions.

After appropriate adjustments, our study does show a slight improvement in our ability to diagnose etiologic factors in women with two or more abortions. This is by a narrow margin. In terms of outcome, we could not show any significant improvement. This is related in part to the restricted number of patients. It may also reflect the poor efficacy of currently available treatments for the newly identified causes.

It is somewhat discouraging to realize how little ground we have gained in this field over the past 10 years. The improved technology has not been matched by an equal diagnostic yield. This underlines the dire need for increased research in this area. Multicenter trials appear to offer the best hope because of their ability to acquire large numbers in a relatively short period of time.

In terms of daily practice, this study reemphasizes the need for a complete workup in all couples with a history of recurrent spontaneous abortions. The finding that the same team can experience such wide variations in the prevalence of etiologic factors should cast an appropriate skepticism in applying the data of any study to one's practice. Instead, large pools of data from several centers appear more appropriate. It is hoped that the next decade will finally see major progress accomplished in this area.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

DR. RAMEZ S. AZOURY, Norfolk, Virginia. This study compares 131 patients (group B) investigated between 1987 and 1991 with another group of 100 patients (group A) investigated between 1968 and 1977, for recurrent abortion in the same academic center. A standardized protocol is followed and the results are compiled retrospectively.

The diagnostic advances made include improved karyotyping with G- and C-banding techniques and ultra-high-banding resolution, laparoscopy replacing gynecography, hysteroscopy used as a complement to hysterosalpingography, a flexible sampling device for sampling the endometrium, urinary LH monitoring, coupled at times with ultrasonographic follicular monitoring, screening for infections, and tests of autoimmune and alloimmune factors suspected to be causes of habitual abortion. The authors stress that at present the diagnosis of autoimmune disease is unreliable, because of variability in laboratory technique throughout the country, and that this obviously needs improvement.

Currently, there are no standardized tests or reporting criteria for the LAC. Dudley and Branch[1] found that there is marked variation in the ability to detect LAC in standardized samples from one laboratory to another and that controversy and personal bias make the task of standardization difficult.

The importance of a complete study to make the etiologic diagnosis is emphasized and the difficulties are apparent. The authors point out the importance of identifying medical disease, thyroid disease and hyperprolactinemia especially. Both of these can interfere with the hormonal milieu during the menstrual cycle, thus inducing ovulatory and luteal phase defects and thereby spontaneous abortions. Chronic illnesses of course, mainly tuberculosis, can do this and can be easily missed.

I would like also to comment that in this study the most common causes of repeated spontaneous abortions seem to be luteal phase defect and associated ovulatory defects. The first report on this condition by Delfs and Jones[2] stated that 35% of the patients studied at The Johns Hopkins Hospital for repeated spontaneous abortions were identified as having such defects. However, Plouffe et al. repeatedly mentioned in their article that uterine anomalies and balanced chromosomal rearrangements are the only two accepted etiologic factors.

The therapy for recurrent losses in group B differs from that of group A. Hysteroscopic resection of uterine septum has replaced metroplasty. Only those women with positive cervical culture for general microbiologic results and chlamydia or a high antibody titer for chlamydia are treated. All couples for whom no cause can be found are treated with postimplantation progesterone, whereas those identified as having a corpus luteum defect are treated with preimplantation progesterone vaginal suppositories, and patients with autoimmune disorders receive either aspirin alone or aspirin and heparin or aspirin and cortisone. However, there are reports about the use of intravenous immunoglobulin for the treatment of recurrent abortions caused by antiphospholipid antibodies.[3] Would the author care to comment about this modality?

What criteria do the authors use to include some conditions under "possible" and others under "probable" in Table II?

Alloimmune factors are mentioned briefly. I would like to ask the author to comment on the current thoughts of his group about this cause. Should we pursue this investigation all the way in a couple where no etiologic factor could be detected? What are your current thoughts about therapy?

Do the authors believe that a male factor should be considered, in view of some anecdotal reports of repeated spontaneous abortions in four women married to the same husband? The experience at the Jones In-

stitute shows that there is a trend for repeated abortions in patients whose husband has poor sperm morphologic characteristics, i.e., <4% normal forms, by means of strict criteria developed by Tiggerberg and his group[4] in South Africa. However, the number is too small to draw a conclusion.

It would have been interesting to "revisit" the patients in group A to obtain a longer follow-up and predict what would happen to the patients in group B if they underwent follow-up for a longer period of time.

This is an excellent article by a group well known for scientific performance and expertise in the area of reproduction. It is disappointing to learn that, although their ability to diagnose the etiologic factors of recurrent abortion has improved, there has not been a perceptible increase in successful therapy for this intriguing problem.

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Dr. Plouffe (Closing). With regard to the autoimmune issue, there are two camps that are evolving and for those of you with a strong interest in the area I would encourage you to look at *The American Journal of Human Genetics*, January 1992, where there is a beautiful editorial by Dr. Gill from Pittsburgh that really crystallizes the whole debate.[1] Dr. Gill was on the autoimmune-immune side and now is on the autoimmune-genetic side. Basically, the concept is that if there is a high level of shared loci in parents this may indeed represent failure of the normal immunologic mechanism in pregnancy. It may be a problem of lack of recognition of the pregnancy as a foreign allograft and lack of elaboration of the blocking antibodies. That is one concept, and under that theory one would want to initiate immune therapy. Another group, though, believes that those are immune markers for genetic markers, and therefore what you are identifying is couples that share many genetic loci that are critical to normal embryonic development and normal life.

In looking at the outcome of pregnancies in parents who share many loci, it is apparent that not only do they have a high incidence of infertility and a higher incidence of recurrent pregnancy loss, but they also have a higher incidence of cancers that are genetically related. Because of this we believe that the autoimmune etiology may be a reflection of shared genetic load. In our center this is our experience. Couples with many shared loci who have had autoimmune therapy have not had much success and those who have tried without autoimmune therapy have been successful after multiple attempts at pregnancy. Therefore the debate continues and we do not have the answers, but it is one area that needs follow-up.

In terms of male factor infertility, we have the same gut feeling as those in Norfolk, Virginia, that this is a factor. We have had some couples over the years in which the man had highly abnormal sperm parameters. I could not establish a threshold level. Our experience is too small to draw any conclusions on this, but it is one area that needs to be looked into, as well as the presence of antisperm antibodies in either the male or the female partner, which has been thought by some groups to be related to recurrent pregnancy loss.

The role of immunoglobulin for autoimmune therapy is extremely interesting; the data are very promising. The multicenter trial in which we were enrolled compared aspirin and heparin versus aspirin and prednisone. We were able to randomize 20 couples and found a small increase in side effects from the aspirin-prednisone therapy compared with the aspirin-heparin therapy. We were not able to show that there was any benefit of one treatment over the other in terms of pregnancy outcome. At that time the NIH determined that because we had higher side effects in the aspirin-prednisone group, the study should be dropped. That was the only effort of which we are aware of a multicenter trial looking at autoimmune disease. It is hoped that the NIH will revise its policy and we will be able to initiate further studies in this very difficult area. Also at that time we could include γ-globulin therapy and immunoglobulin therapy as part of the study trial.

With regard to the question about possible or probable etiologic factors, we have tried to be as objective as possible. The reason that chromosomal anomalies are recognized by all is that one can look at the outcome. It is possible to look at the fetus and document unbalanced translocations. In looking at uterine anomalies, most people agree, although we do not have a randomized trial of couples treated and not treated with uterine anomalies. We strongly believe that luteal phase defect falls into the known causes of recurrent loss, but we know that other people, including Dr. Grimes, would debate the issue. Therefore we tried to include those factors for which there is a consensus that they are definite causes. Probable causes and those that are hotly debated get more into the issue of possible causes.

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Electroejaculation in combination with in vitro fertilization and gamete micromanipulation for treatment of anejaculatory male infertility

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OBJECTIVE: Failure to ejaculate may be overcome by use of electroejaculation. However, such semen samples are often unsuitable for therapies like intrauterine insemination. The combination of electroejaculation with in vitro fertilization, including gamete micromanipulation, should improve chances of fertilization and pregnancy in such cases.

STUDY DESIGN: Within a private infertility clinic electroejaculation in combination with intrauterine insemination was carried out in 18 cycles (10 couples). Four couples went on to receive therapy by electroejaculation plus in vitro fertilization, along with six other couples (15 cycles total) with semen too poor for intrauterine insemination.

RESULTS: One term pregnancy arose in the electroejaculation–intrauterine insemination group, and one term pregnancy plus one continuing pregnancy arose from two couples (three cycles) who underwent in vitro fertilization with conventional insemination after electroejaculation. Six couples (nine cycles) had embryos arising only from gamete micromanipulation transferred, and this yielded two term pregnancies, one spontaneous abortion, and a biochemical pregnancy. Two couples (three cycles) failed to achieve fertilization even with micromanipulation; however, donor-inseminated eggs gave rise to two term pregnancies and one continuing pregnancy in these patients.

CONCLUSIONS: This report confirms the feasibility of in vitro fertilization in conjunction with electroejaculation and extends the therapy to incorporate gamete micromanipulation. (AM J OBSTET GYNECOL 1992;167:322-6.)

Key words: Electroejaculation, anejaculatory infertility, gamete micromanipulation

The combination of electroejaculation and intrauterine insemination has given rise to several reports of successful pregnancies for couples in whom the male partner suffers from paraplegic anejaculation.[1,2] However, it is common in such men for spermatozoal counts to be low and progressive motility to be poor, possibly because of either testicular hyperthermia or negative influences of the electroejaculation procedure on semen production. For example, electroejaculation may not initiate a full ejaculatory response.[3] These factors militate against success with timed intrauterine insemination alone, and Ayers et al.[4] reported the first successful application of in vitro fertilization (IVF) and embryo transfer in combination with electroejaculation in a case of oligoasthenozoospermia. Recently gamete micromanipulation procedures have been developed to enhance fertilization during IVF for severe male factor infertility,[5,6] and such approaches may be appropriately applied to the poorer cases of electroejaculation–IVF.

In addition to performing electroejaculation in combination with intrauterine insemination, we have also adopted IVF in cases untreatable by intrauterine insemination or after repeated failure with electroejaculation–intrauterine insemination. In several cases where spermatozoal performance was particularly poor, we have undertaken partial zona dissection[7] and in three cases subzonal spermatozoal insertion[8] in an attempt to improve chances of fertilization during IVF.

Material and methods

Patients. Ten women underwent 18 intrauterine insemination cycles with spermatozoa previously collected by electroejaculation. One other woman whose partner's semen was inadequate for intrauterine insemination on two occasions underwent insemination with frozen-thawed donor semen at the second failed electroejaculation attempt. All naturally ovulatory women received one intrauterine insemination midcycle according to ovulation estimates, on the basis of a urinary luteinizing hormone surge, except two women in whom

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Table I. Seminal profile of 10 couples who underwent electroejaculation plus intrauterine insemination (18 cycles)

Semen source	*Antegrade*	*Retrograde*
Prepreparation (average ± SD)		
Diluted volume (ml)	8.9 ± 7.0	50.1 ± 38.2
Count (million/ml)	18.0 ± 31.1	8.5 ± 15.0
pH	6.9 ± 0.2	6.6 ± 0.3
Motility (%)	12.4 ± 12.7	8.1 ± 7.9
Progression (0-4)	1.7 ± 0.7	1.5 ± 0.8
Contamination	72%*	83%*
Intrauterine insemination specimen postpreparation (average ± SD)		
Volume (ml)	0.7 ± 0.3	
Total motile (million)	13.4 ± 43.2	
Progression (0-4)	2.6 ± 0.4	
Outcome		
Term pregnancy	1†	

*All samples.
†One male infant per 18 cycles of intrauterine insemination.

intrauterine insemination was performed twice midcycle.

Of these 10 couples, four subsequently undertook treatment by IVF (five cycles). A further six couples underwent IVF–electroejaculation treatment directly (10 cycles) because of poor spermatozoal quality. Male partners all were anejaculatory, one being quadriplegic and seven paraplegic after spinal cord injury. The other two men had postoperative damage subsequent to retroperitoneal lymph node dissection. Six of the female partners also had secondary complicating infertilities including anovulation, mild endometriosis, adhesions, polycystic ovarian syndrome, microadenoma and mild endometriosis, and fibroids. Average ± SD age of these 10 women at the time of treatment was 34.3 ± 2.9 years. A variety of ovulation induction protocols was used to achieve superovulation. In four cycles a combination of clomiphene citrate and human menopausal gonadotropin was used, and in one cycle human menopausal gonadotropin only was used. There were nine cycles of long-protocol leuprolide acetate treatment coupled with pure follicle-stimulating hormone and human menopausal gonadotropin, and one cycle used a short-protocol leuprolide acetate regimen in combination with follicle-stimulating hormone and human menopausal gonadotropin. Ovulation was triggered in all cases with human chorionic gonadotropin when estradiol rise and follicular growth patterns were optimal. Additionally, all women received midcycle corticosteroids and antibiotics[9] in anticipation of transfer of embryos with perforated zonae pellucidae.

Electroejaculation. With the male partner in a lateral decubitus position, anoscopy was performed to ascertain that there were no rectal hemorrhages, mucosal ulcerations, or neoplasms. Next urethral catheterization was carried out to drain the bladder, and the bladder was refilled with approximately 30 to 60 ml sterile Hepes-buffered minimum essential medium with 2.5% bovine serum albumin before removal of the catheter. The electroejaculator was inserted atraumatically into the rectum, and an electroejaculatory stimulation of 5 to 35 V and 100 to 600 mA (sine wave–alternating current) was delivered. All antegrade ejaculation was collected in a sterile specimen container and immediately examined for the presence of spermatozoa. At the end of the procedure the man again underwent anoscopy to check for any rectal lesions, and the bladder was recatheterized to obtain any retrograde ejaculation. Blood pressure was monitored throughout the procedure.

Intrauterine insemination, IVF, partial zona dissection, and subzonal spermatozoal insertion. Semen samples were prepared by washing and sedimentation under mineral oil[10] or by a mini-Percoll technique.[11] Pentoxifylline (2.5 mmol/L) and 2′-deoxyadenosine (2 mmol/L) were used in the spermatozoal preparation for subzonal spermatozoal insertion.[12] Intrauterine insemination was performed with a Wallace embryo replacement catheter (H.G. Wallace, Colchester, United Kingdom) placing between 0.25 and 1.0 ml processed spermatozoal suspension close to the uterine fundus. IVF, partial zona dissection, and subzonal spermatozoal insertion procedures were performed as previously described.[7, 8] Embryo transfers were performed with a Wallace catheter, and all patients received luteal phase support as either human chorionic gonadotropin on days 2 and 4 after the oocyte retrieval or progesterone daily until pregnancy testing.

Results

Table I indicates the averaged seminal profile for both antegrade and retrograde ejaculates before processing for 18 cycles of intrauterine insemination. Because of the need to dilute samples, volumes collected are relatively large and vary according to the ease of collection. Likewise spermatozoal counts vary radically.

Table II. Averaged seminal profile (antegrade and retrograde), fertilization, and pregnancy outcome in 10 couples (15 cycles) who underwent electroejaculation plus IVF

	IVF insemination strategy						
	Conventional only	*Conventional–partial zona dissection–subzonal spermatozoal insertion*			*Partial zona dissection–subzonal spermatozoal insertion–donor*		
No. of cycles	3	9			3		
Volume (ml)	31.3 ± 22.7	15.3 ± 7.0			32.2 ± 44.6		
Count (million/ml)	7.6 ± 4.1	27.5 ± 29.5			2.1 ± 2.3		
pH	7.2 ± 0.05	7.5 ± 0.5			6.9 ± 0.1		
Motility (%)	18.6 ± 14.9	6.6 ± 9.6			7.4 ± 3.9		
Progression (0-4)	1.8 ± 0.6	2.0 ± 1.3			1.1 ± 0.9		
Fertilization	21/34	1/13	11/36	6/18	4/5	0/4	0/1
	Conventional	Conventional	Partial zona dissection	Subzonal spermatozoal insertion	Donor	Partial zona dissection	Subzonal spermatozoal insertion
Cryopreservation	12	—	—	1*	—	—	—
Embryo transfer	9		9	5	4		
Outcome							
Delivered	1		2			2	
Continuing	1		—			1	
Other	—		2†			—	

*Five-cell embryo frozen after reinsemination by subzonal spermatozoal insertion.

†One spontaneous abortion and one biochemical pregnancy.

Progressive motility is low in all cases, and >70% of all samples collected contained considerable contamination, particularly blood cells and nondescript cellular debris. One pregnancy with delivery of a male infant arose from these 18 cycles, and interestingly it was from one of the two cycles in which insemination was carried out twice midcycle, the total motile spermatozoal counts being 6 and 17 million on these occasions.

Table II considers the combined antegrade and retrograde seminal profiles in the 15 electroejaculation–IVF cycles undertaken. Spermatozoal counts and progressive motility were low in all cases, especially in the three cycles where fertilization failed with husbands' spermatozoa, although oocyte numbers were low in these cases. In two couples (three cycles) conventional insemination of oocytes alone was carried out with a 62% fertilization rate, 12 embryos were cryopreserved, and nine embryos were transferred to give one pregnancy with delivery of a female infant and one continuing pregnancy. In nine cases conventional insemination was also backed up by partial zona dissection and subzonal spermatozoal insertion in a greater proportion of the oocytes collected. One fertilized oocyte from the conventional group (7.7% fertilization rate) did not develop for transfer, whereas 11 partial zona dissection oocytes were fertilized (30.6% fertilization rate) and nine cleaved to be transferred to give two pregnancies with delivery of male infants, one spontaneous abortion at 10 weeks of pregnancy, and a biochemical pregnancy. Two oocytes unfertilized by partial zona dissection underwent reinsemination by subzonal spermatozoal insertion, and one fertilized to be frozen as a five-cell embryo. In two cycles fertilization arose only from subzonal spermatozoal insertion with a 31.2% fertilization rate (5/16). All embryos were transferred but without pregnancy. Finally, in two couples (three cycles) in whom fertilization was not achieved with the husband's spermatozoa a total of four embryos (80% fertilization rate) from donor insemination were transferred to give one pregnancy with delivery of a male infant, one pregnancy with delivery of a female infant, and one continuing pregnancy.

Comment

The inability to achieve seminal ejaculation can in many instances be overcome by the use of electroejaculation. This procedure, however, does not in all cases provide semen of sufficient quality for use in intrauterine insemination. Where semen quality is poor, IVF can be adopted to enhance chances of fertilization and pregnancy,[4] and our results here confirm that as a realistic course of treatment. In addition, chances of fertilization may be further improved by the implementation of some form of gamete micromanipulation, especially when seminal quality is actually suboptimal, even for use in conventional IVF. Again the few cases reported here seem to bear this out, with three pregnancies being initiated and two male infants delivered from the six cycles in which embryos arose from partial zona dissection micromanipulated oocytes. Subzonal spermatozoal insertion reinsemination of two oocytes that failed to fertilize after conventional insemination

gave rise to one late-fertilized cleaved embryo that was frozen for later, more synchronous transfer after thawing.[13] Further, subzonal spermatozoal insertion in two cycles generated five embryos for two transfers, but pregnancy did not result.

As previously found,[7] no one particular seminal characteristic was clearly predictive of the need for gamete micromanipulation. There was, however, a suggestion that higher motility was associated with greater ease of fertilization in IVF, because this was highest in those two patients who only required conventional insemination, although this difference was not statistically significant. It is known that systemic antispermatozoal autoimmunity can occur in spinal cord–injured men,[14] and it is conceivable that this factor may have had some bearing on the poor spermatozoal motility and low fertilization outcome in some of the cases reported here. However, antispermatozoal antibodies were not assayed in this instance because of insufficient sperm numbers, and efforts were concentrated on obtaining sufficient spermatozoal suspension for insemination.

Continuing improvement in many areas of reproductive technologies underline the need for our constant reappraisal of what is possible in infertility therapy. Electroejaculation has enabled spermatozoa to be routinely collected from men previously thought of as untreatable. Gamete micromanipulation has allowed fertilization to be realized in cases of severe male factor infertility, otherwise considered untreatable by IVF. Indeed, further improvement of these latter techniques may eventually allow us to achieve fertilization in those cases where we still have had to resort to donor insemination when spermatozoal counts were too low, and progressive motility too feeble, or when oocyte numbers were limited. In any event we have established that electroejaculation of anejaculatory men in combination with IVF with or without gamete micromanipulation is a viable therapeutic approach.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

Dr. R. G. Brame, Greenville, South Carolina. This treatise addresses a topic that is rather unique but is an important subject, not only because it addresses the reproductive needs of a seriously impaired segment of the population but it also brings to our attention, again, some of the modern technology that may be brought to bear on the reproductive failures of the many others not so handicapped.

First, let me reemphasize some observations already made by the presentor: the women partners of these men suffered an unusually large number of concomitant reproductive problems, an interesting finding for which no explanation is apparent. Another observation: electroejaculation is a new technology, but early in its history rectal injuries could be caused both by direct trauma and electric shock. It appears now that those problems have been resolved and electroejaculation can be carried out without even minor complications for the male. Further, it is fairly obvious that neurologic impairment in the male, however mediated, results in poor semen quality. There are small numbers of sperm, poor motility, and contamination of the already poor samples by much cellular debris and blood.

I did struggle, Dr. Toledo, a great deal with some of your math. I can add up seven pregnancies in your abstract but only six that were conceived by IVF. I have made the presumption that you included the one pa-

tient who had intrauterine insemination. I have no problem with this except that it is not totally clear that that is the case, first, and second, I'm not sure why the patient having intrauterine insemination was included in the series, given the title. In addition, you speak to the issue of enhancing IVF by the addition of partial zona dissection, yet when I examine your tables, I find conventional IVF to have a 67% fertilization rate whereas conventional-partial zona dissection has a 28% rate and partial zona dissection with donor eggs has a 0% fertilization rate. If I have obviously misinterpreted your figures, as I'm guessing that I have, I'll be grateful for your help. Also, I did not identify in your data a recounting of how many IVF implants were made in each patient or over what amount of time. But none of this detracts from your success.

You do present an apparently effective approach to the loss of reproductive capacity in individuals who have experienced spinal cord or other neurologic injury, and although it is a problem that affects only a small number of patients, it is an important step in our attempts to make the benefits of modern medicine available to the entire spectrum of the handicapped.

Dr. Roger P. Smith, Augusta, Georgia. Would the authors please comment on whether they use alternating or direct current? If they use alternating current, would they comment on wave form and duty cycle?

Dr. Barry S. Verkauf, Tampa, Florida. We have been fortunate to have two of our first six patients seen with clomiphene citrate intrauterine insemination with electroejaculation. I like to ask Dr. Toledo several questions relative to handling the ejaculate. First, since there is a propensity to infection, were antibiotics used for both the male and female? Second, because antegrade specimens are not always obtained, was alkalinization of the urine done? Third, because experience around the country is quite limited with this technology, did you notice any improvement in the quality of the ejaculate with the number of electroejaculations that individual men were exposed to?

Dr. Toledo (Closing). In answer to Dr. Brame's question regarding the actual pregnancies, we included biochemical pregnancies as the seven, not the intrauterine insemination. The seven total pregnancies were in that particular group. Basically, we had a total of two cycles with a particular type of technique where we felt that initially the insemination specimen quality particularly with regard to motility was improving. Granted, 22% motility is not what we would consider normal, but we felt that particular percentage was going to be sufficient in allowing for conventional insemination. We inseminated all four of these oocytes that I showed you with conventional techniques using micromanipulation, obtaining roughly a 62% fertilization rate resulting in the delivery of one term pregnancy. Other pregnancies were a combination of two groups. The conventional group did not contribute because, although we did get one oocyte fertilized, there was no subsequent cleavage resulting in a nonviable embryo. All of these pregnancies resulted from partial zona dissection, giving about a 30% fertilization rate. In the subzonal insertion group, again as I emphasized, was not one where we were able to replace the embryo because of the time lag it is still in cryopreservation. In this particular group, the two delivered pregnancies were all an off-shoot of donor backup. I apologize for the confusion in the numbers.

In answer to the question regarding the current, I believe that it was alternating current with a sine waveform.

With regard to the collection of the ejaculate, we did cover both the female and her husband with antibiotics. Whenever we manipulate the zona pellucida, we are concerned about exposing the embryo to infection; therefore we do use antibiotic coverage in both the woman and her husband.

The husband's urine was alkalinized before the procedure. This is usually done with a sodium bicarbonate preparation.

With regard to the overall improvement of the semen specimen with repeated ejaculation, the answer was that it was very variable. We saw no consistent pattern. It was totally unpredictable. West of Houston has also pointed out that the response to electroejaculation is quite variable and is frustrating to those undergoing the treatment.

If we had to do it over again for the poor responders, we would automatically use our experience with primary subzonal insertion. The best shot at the oocyte in terms of fertilization is first-day exposure in the hope that donor backup would not have to be relied on.

Red blood cell transfusion and cesarean section

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OBJECTIVE: Our objective was to determine the incidence of blood transfusion in patients delivered by cesarean section and to identify factors predictive of the need for transfusion.
STUDY DESIGN: A chart review was performed on cesarean patients receiving blood transfusion over a year at the Medical College of Virginia Hospital and an equal number of randomly selected cesarean patients.
RESULTS: Sixty-one (6.8%) patients received a packed red blood cell transfusion intraoperatively or postoperatively. Transfusion was associated with lower gestational ages, antepartum bleeding, arrest of descent, and longer postoperative stays. After stepwise logistic regression analysis, only antepartum bleeding and preoperative hemoglobin were significant independent predictors of the need for blood transfusion.
CONCLUSION: Transfusion with cesarean section is common and is associated with antepartum bleeding and other primarily intrapartum and unpredictable factors. (Am J Obstet Gynecol 1992;167:327-32.)

Key words: Blood transfusion, autologous donation, cesarean section

Patient concerns about receiving a blood transfusion have progressively increased in the past decade, due primarily to concerns about infectious complications, specifically hepatitis and human immunodeficiency virus infections. In some preoperative patients, the risk of infectious complications can be minimized by the use of preoperative autologous blood transfusions. Blood donation for autologous transfusion has been performed in the third trimester of pregnancy without major complications.[1-3] However, in contradistinction to many other surgical patients, it is difficult to predict which obstetric patients will need blood transfusions. A recent retrospective study of over 30,000 deliveries reported that 2.6% of obstetric patients received ≥1 U of blood.[4] Patients at increased risk for transfusion included those with more than one prior abortion or a multiple gestation, patients undergoing cesarean section, particularly a primary cesarean section, and patients with abnormalities in placentation and amniotic fluid volume. Patients who received a transfusion also had a significantly lower gestational age and neonatal birth weight and a significantly higher estimated blood loss. However, many of these factors are closely interrelated, and no regression analysis was performed.

Because one group at relatively high risk for hemorrhage requiring transfusion consists of patients undergoing cesarean section,[4-6] our aim was to describe those patients requiring blood transfusion at the time of cesarean section in an attempt to identify factors that might predict a need for transfusion. In addition, we wished to determine whether those patients who underwent blood transfusion were at increased risk for prolonged hospital stay and other postoperative complications.

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Material and methods

All patients discharged between July 1, 1988, and June 30, 1989, with the diagnosis of cesarean section were identified and their blood bank records reviewed. Those patients who were identified as having received blood products during their hospitalization constituted our study group. To create a comparison group, we matched each transfused patient with the next cesarean section patient on the alphabetic discharge list in the same time period who did not receive a transfusion. We reviewed the hospital charts for these patients and extracted data on patient demographics, obstetric and medical history, type of cesarean section performed, indications for surgery, anesthesia, preoperative and postoperative blood counts, estimated blood loss at the time of surgery, postoperative complications, and postoperative stay. Timing of the transfusion in relation to surgery and vital signs at the time of transfusion were also recorded for the study group.

Statistical analysis was performed with True Epistat and SAS statistical packages. Fisher's exact test and χ^2 analysis were used, as appropriate, for discrete variables. The Student t test was used for continuous variables. The stepwise logistic regression analyses were performed with SAS software. A p value of ≤ 0.05 was considered significant.

Table I. Comparison of characteristics of mothers and babies delivered by cesarean section with and without blood transfusion

Variable	*Blood transfusion (n = 61)*	*No blood transfusion (n = 69)*
Age (yr)	25.6 ± 6.1	25.0 ± 6.0
Nulliparous (No.)	26 (43%)	29 (42%)
Ratio abortions to deliveries	0.21 ± 0.26	0.13 ± 0.20
Prior cesarean section (No.)	17 (28%)	19 (28%)
Gestational age (wk)	35.3 ± 5.1	37.0 ± 4.1
Birth weight (gm)*	2456 ± 1150	2696 ± 883
Intrauterine growth retardation* (No.)	4/64 (6%)	8/76 (11%)

Values represent mean ± SD.

*Includes 10 sets of twins.

Results

Eight hundred ninety-nine patients were identified as having undergone cesarean section during this time period. Review of blood bank records revealed that 69 received blood products. Analysis of the hospital charts revealed that two patients in the study group did not, in fact, receive blood products. An additional two patients only received blood preoperatively, and four patients were transfused with platelets or fresh-frozen plasma but did not receive packed red blood cells. These eight patients were eliminated from further analysis; thus our study group comprised 61 patients (6.8% of all cesarean sections) who received packed red blood cells intraoperatively or postoperatively. Thirty-nine (64%) received the transfusion intraoperatively or immediately postoperatively.

There were no significant differences between the two groups in maternal age, incidence of prior cesarean section, neonatal birth weight, or intrauterine growth retardation (Table I). Although there was no significant difference between the two groups in gravidity, parity, or the number of prior abortions, there was a significant difference in the ratio of abortions to deliveries. The mean gestational age at the time of delivery was significantly lower in the transfused group, although the difference in the number of patients delivered prematurely (<37 weeks) was not statistically significant (51% vs 36%, p not significant).

Indications for cesarean section delivery included elective repeat cesarean section, active phase arrest, fetal distress, placenta previa, arrest of descent in the second stage, and abnormal presentation. Both placenta previa and arrest of descent were significantly more often cited as indications for cesarean section in the study group (Table II). There was no difference between the two groups in the frequency of repeat cesarean sections after a trial of labor (12% vs 10%).

Table II. Comparison of cesarean section characteristics in patients with and without blood transfusion

Variable	*Blood transfusion (n = 61)*		*No blood transfusion (n = 69)*		*p Value*
	No.	*%*	*No.*	*%*	
Indication for cesarean section					
Elective repeat cesarean section	6	10	9	13	NS
Arrest of labor	7	12	16	23	NS
Fetal distress	16	26	16	23	NS
Placenta previa	8	13	0	0	0.002
Arrest of descent	8	13	2	3	0.045
Abnormal presentation	9	15	18	26	NS
Other	4	7	5	7	NS
Type of cesarean section					
Low transverse	41	67	65	94	0.004*
Vertical	14	23	4	6	0.005*
Cesarean hysterectomy	6	10	0	0	0.009*
Anesthesia					
General	32	53	20	29	0.007**
Epidural	21	34	39	57	0.014**
Spinal	3	5	6	9	NS
Other	5	8	4	6	NS
Preoperative hemoglobin (gm/dl, mean ± SD)	10.8 ± 1.7		11.6 ± 1.4		0.005

NS, Not significant.

*When compared with all other types of cesarean section combined.

†When compared with all other forms of anesthesia combined.

Transfused patients were more likely to have a vertical uterine incision or cesarean hysterectomy and to have undergone general anesthesia.

As shown in Table III, placenta previa and third-trimester bleeding from causes other than placenta previa were significantly more common in the study group. There were no significant differences in the frequency of other antepartum complications, including chronic hypertension, diabetes, multiple gestation, and preterm labor.

There were no significant differences in the occurrence of preeclampsia, chorioamnionitis, and disseminated intravascular coagulation. Not unexpectedly, atony occurred significantly more frequently in the transfused patients, and the average estimated blood loss at the time of surgery was also greater, with 53 (87%) having an estimated blood loss >1000 ml, compared with 29 (42%) in the nontransfused patients ($p < 0.0001$). Although patients receiving blood had a longer mean postoperative stay, there was no difference between the two groups in the incidence of infectious complications or wound breakdown (Table IV). The number of patients with prolonged postoperative stays (≥5 days) was not significantly different (73% vs 58%, $p = 0.067$). In spite of transfusion, on the average, the

Table III. Comparison of complications associated with pregnancy and delivery in patients with and without blood transfusion

Variable	*Blood transfusion (n = 61)*		*No blood transfusion (n = 69)*		*p Value*
	No.	*%*	*No.*	*%*	
Antepartum					
Chronic hypertension	2	3	6	9	NS
Diabetes	4	7	6	9	NS
Multiple gestation	4	8	7	10	NS
Third-trimester bleeding*	13	21	3	4	0.003
Placenta previa	7	12	0	0	0.004
Preterm labor	18	30	13	19	NS
Intrapartum					
Preeclampsia	11	18	9	13	NS
Chorioamnionitis	12	20	12	17	NS
Disseminated intravascular coagulation	2	3	0	0	NS
Atony	12	20	0	0	<0.0001
Estimated blood loss (ml, mean ± SD)	1468 ± 706		870 ± 198		<0.0001
Estimated blood loss >1000 ml	53	87	29	42	<0.0001

NS, Not significant.
*Not including placenta previa.

Table IV. Comparison of postoperative course in patients with and without blood transfusions

Variable	*Blood transfusion (n = 61)*	*No blood transfusion (n = 69)*	*p Value*
Postoperative stay (days, mean ± SD)*	6.1 ± 3.9	5.0 ± 1.5	0.032
Postoperative stay ≥5 days* (No.)	44 (74%)	40 (58%)	NS
Endometritis (No.)	6 (10%)	10 (15%)	NS
Wound breakdown (No.)	11 (18%)	6 (9%)	NS
Total infectious complications (No.)	19 (31%)	17 (25%)	NS
Lowest hemoglobin (gm/dl, mean ± SD)	7.6 ± 1.4	10.0 ± 1.4	<0.0001
Discharge hemoglobin (gm/dl, mean ± SD)	9.4 ± 1.2	10.0 ± 1.3	0.004
Change in hemoglobin, preoperative to discharge (gm/dl, mean ± SD)	1.4 ± 2.2	1.7 ± 1.4	NS

NS, Not significant.
*Does not include one patient with thrombocytopenic purpura who had a postoperative stay of 57 days.

Table V. Characteristics of patients receiving a blood transfusion

At time of transfusion	
Heart rate (beats/min, mean ± SD)	100 ± 20
Heart rate >100 beats/min	40%
Systolic blood pressure (mm Hg, mean ± SD)	121 ± 23
Systolic blood pressure <100 mm Hg	13%
Diastolic blood pressure (mm Hg, mean ± SD)	69 ± 15
Diastolic blood pressure <60 mm Hg	25%
Units transfused* (mean ± SD)	2.8 ± 1.4
Received >2 U packed red blood cells*	35%

*Does not include one patient who received 21 U of packed red blood cells in association with treatment post partum for thrombotic thrombocytopenic purpura.

study group was discharged with a lower hemoglobin, although severe anemia at discharge (hemoglobin <8 gm/dl) was uncommon in both groups.

The hemodynamic parameters at the time of packed red blood cells transfusion were generally not indicative of shock (Table V). Twenty-one patients (35%) received more than two units of packed red blood cells; the mean number of units transfused was 2.8 ± 1.4. For patients with placenta previa, the mean number of units transfused was 3.2 ± 2.0 with a range from 2 to 7 U.

A stepwise logistic regression analysis was performed with variables with a p value of ≤0.1 on univariate analysis. These included ratio of abortions to deliveries, gestational age, cesarean section type, anesthesia, nonprevia antepartum bleeding, preoperative hemoglobin, and estimated blood loss. Only gestational age, bleeding from causes other than placenta previa, and estimated blood loss remained as significant predictors of the need for blood transfusion. Although the presence of placenta previa was strongly associated with the need for transfusion, it could not be used in this analysis because of its absence in the control group.

Because these variables cannot be used as predictors for the use of autologous transfusion, the analysis was then repeated with only those variables that might be useful predictors for the purpose of autologous donation. For this analysis, placenta previa was combined with other causes of antepartum bleeding. The presence of a low preoperative hemoglobin and antepartum bleeding (from any cause) remained significantly associated with the need for blood transfusion.

Comment

The frequency of blood transfusion in association with cesarean section in our population was 6.8%,

higher than some reports[5,6] and similar to others.[4] This relatively high rate of transfusion was surprising, especially given the fact that many obstetricians report decreasing their frequency of transfusion in the last 5 years (unpublished data). The frequency of transfusion may be partially explained by two factors. First, in a large urban hospital training program the surgeons are less experienced than those in some other hospitals. More importantly, this hospital serves as a tertiary referral center and treats a large proportion of indigent and high-risk patients. The decision to perform transfusion was made on a case-by-case basis by the physicians (faculty and senior house officers) caring for the patient.

The factors associated with blood transfusion in this study are remarkably similar to those found by Klapholz.[4] This includes the obstetric history, gestational age, birth weight, estimated blood loss, presence of antepartum bleeding, and abnormal placentation. After logistic regression analysis, however, many of these risk factors are eliminated.

Predictably, patients who ultimately required blood transfusion were more likely to have had preoperative bleeding complications, and as a result their preoperative hemoglobin was significantly lower. An increased incidence of blood transfusion after cesarean section for vaginal bleeding was also noted by Hill and Lavin,[6] who found no other associated risk factors. Rather than simply being a risk factor for transfusion, the presence of antepartum bleeding and a low preoperative hemoglobin may also lower the physician's threshold for initiating transfusion.

Vertical uterine incisions, cesarean hysterectomy, and the use of general anesthesia, all of which have been associated with increased intraoperative blood loss,[7,8] were more common in the transfused group. Rather than causing increased blood loss, these findings may instead reflect the emergent and complicated nature of many cesarean sections associated with excessive intrapartum bleeding.

Of interest is the fact that the only antepartum complications that were significantly increased in the transfused group were third trimester bleeding and placenta previa. The ability to predict that these patients might need a blood transfusion at the time of cesarean section does not necessarily allow autologous transfusion because patients with preexisting anemia and those at high risk for spontaneous hemorrhage are generally not acceptable candidates for autologous donation. Some authors, however, suggest that the diagnosis of placenta previa is an indication for autologous donation.[2,9] Whether there may be a subgroup of asymptomatic patients with placenta previa who might benefit from autologous transfusion requires further study. No other factor, including previous cesarean section, was significantly more common in the transfused group.

We conclude that although red blood cell transfusion associated with cesarean section delivery is frequently performed, it is difficult to predict those patients who would most benefit from autologous donation. This is primarily due to inability to (1) predict which patients will require cesarean section delivery, (2) predict which cesarean section patients will require blood transfusion, and (3) safely perform autologous donation in those patients at highest risk. As a result, the role of autologous donation and transfusion in the care of obstetric patients remains to be defined.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

DR. STEPHEN R. GOLDMAN, Atlanta, Georgia. Although many of the traditional risks of blood transfusion have been reduced by improved blood banking technology, a number of risks still remain. The recent introduction of testing for so-called hepatitis C may further decrease, but will not likely eliminate, the risk of posttransfusion hepatitis, which currently has been variously quoted between 1 and 100 and 1 in 300 for each unit transfused.[1] The risk for transfusion-acquired human immunodeficiency virus is much smaller than that for hepatitis, at 1 in 40,000 to 1 in 100,000, but mortality from the disease is presently considered 100% and donor screening by demographic risk factors may become less efficient if, as currently suggested, human immunodeficiency virus infection continues to increase in the heterosexual, non-drug-abusing population.

Under the circumstances, the use of autologous blood in obstetric patients must be investigated. Dickason and Dinsmoor's study has clearly shown the difficulty we can face when we try to prospectively identify the population at risk for transfusion, for whom autologous donation might be desirable. In this study, 6.8% of patients undergoing cesarean section required transfusion. By way of comparison and with due respect to obvious differences in respective settings, at Piedmont Hospital in Atlanta where I practice, the incidence of transfusion for patients undergoing cesarean section was 1.45% from data accumulated between 1984 and 1986 (personal communication). This almost fivefold difference raises some questions about transfusion practice in the current study. Did any of the patients receive autologous units? How many were single-unit transfusions? Were the cases of transfusion, in general, reviewed for appropriateness? If these factors were not analyzed, and had they been, it is possible that the results of the study would have been quite different.

A number of other concerns about autologous transfusions need to be addressed, beginning with whether autologous donation in pregnancy is safe. On the basis of the studies cited in this article and on one other I was able to locate,[2] it would seem that it is safe. However, the numbers of patients involved are relatively small, and all were confined to the latter portion of the third trimester.

Can autologous donation be done without fetal monitoring? That situation might be necessary if it is to be a practical modality. There is one study by McVay et al.[3] that shows no increased untoward effects in such a group.

In Dickason and Dinsmoor's study, one risk group, those with low preoperative hemoglobin values, presents a "catch 22" with respect to autologous donation. If the hemoglobin value is sufficiently low, the patient is not a candidate for donation, but if the value is marginal, it may be rendered low by the act of donation. In McVay et al.'s study, transfusion incidence was 21% for women donating within 1 week of delivery versus 9.4% for women donating earlier.

If one excludes patients with acute third-trimester hemorrhage necessitating immediate cesarean section, the remaining patients at risk for bleeding, namely, those with placenta previa, may be well served by autologous donation, provided they are not having recurrent episodes of bleeding resulting in marginal anemia contraindicating donation. However, the safety of multiple unit donations would need to be established.

The article mentions vertical incision and cesarean hysterectomy as conditions significantly associated with transfusion. The occasional situation in which either circumstance might be known in advance could well be indications for autologous donation. In the Piedmont Hospital series, 5% of patients undergoing cesarean section required vertical incision, whereas vertical incisions accounted for 10% of those transfused.

Although not identified in the current study, one other group of patients who might also benefit from autologous donation are those individuals with multiple red blood cell–associated atypical antibodies, for whom it would be highly difficult or impossible to find compatible homologous blood, especially in an emergency.

Finally, it should be duly noted that transfusion risks can also be prevented by transfusion avoidance. The 1988 report from the National Institutes of Health, among others, points out current concepts of transfusion based on clinical assessment and relegates outdated concepts of various ancillary benefits of transfusion to medical history. The role of ultrasonography in transfusion avoidance should also be explored. Placental localization before planned cesarean section may well be desirable. Antepartum localization in patients with risk factors for cesarean section or transfusion would be worth considering if studies are done that establish predictive criteria. Intrapartum localization scan just before cesarean section is an alternative to be considered, especially if predictive criteria turn out not to exist. However, the rationale for all or any of this probably depends, as much as anything else, on the overall incidence of indicated homologous transfusion in one's institution or practice.

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Dr. Robert B. Hunt, Boston, Massachusetts. I am amazed at the amount of blood that patients can lose at cesarean section. As was pointed out, there are problems with autologous blood transfusions. First, a lot of the blood is not used; second, the patients who need it most usually cannot give it; third, it is often not possible to predict in advance who will lose a lot of blood. Might the solution lie with expanded use of the cell saver? We have used it quite effectively in our patients undergoing multiple myomectomy.

Dr. William Mann, Newport News, Virginia. When we look at the factors associated with blood loss, it appears that the two most likely to be controlled by the surgeon are the choice of anesthetic and the intraoperative blood loss, yet the choice of anesthetic is often dictated by the situation. I wonder then, because intraoperative blood loss is the only parameter over which the surgeon has control, if the data might be affected by the use of the stapling device in making the uterine incision. The staple might be associated with a longer operating time; therefore I wonder if operating time affected blood loss.

Dr. Leslie W. Beadling, Jacksonville, Florida. What were the criteria for blood transfusion?

Dr. Dinsmoor (Closing). I agree that our transfusion rate was somewhat high. One of the reasons we did the study was to determine this. Patients being scheduled

for elective repeat cesarean often asked what their chance of receiving a transfusion might be and until this time I had no numbers to give them. I think that there are two reasons for our relatively high transfusion rate: (1) We have a large training program and therefore we have some less experienced surgeons on some of the cases; (2) more important, we are a tertiary referral center and are referred a lot of patients who are extremely high risk and already having bleeding problems. I would note that the study by Dr. Klapholz, who reported on 30,000 patients from the Beth Israel Hospital, cited a transfusion rate in the cesarean patients of 5.2%.

We did not use any autologous units in this study. It is not our routine to do so. We are thinking about studying this in our placenta previa patients.

There was one single-unit transfusion in these 61 patients. Yes, all of our transfusions are reviewed by a blood utilization committee.

I have been informallly observing our placenta previa patients to see how many might have been candidates for autologous transfusions. Many cases are diagnosed when the patients come in bleeding and are delivered that same day. In the three patients we have had in recent months who have had the diagnosis made antepartum, one was too anemic to donate, one was too late in her pregnancy to donate, and the third was too early in her pregnancy to donate. The last patient may be our first candidate in this group.

It is my understanding that the cell saver cannot be used in obstetric patients because the blood is going to be contaminated with amniotic fluid. To infuse blood that contains amniotic fluid runs the risk of amniotic fluid embolism–type complications. With regard to the choice of anesthesia, oftentimes the emergent nature of the cesarean section determines the choice of anesthesia. I have a suspicion that our estimated intraoperative blood loss is biased in our patients who received transfusions. Many received intraoperative transfusions, and the amount of intraoperative blood loss and number of units of blood that the patient received intraoperatively might have had a significant influence on the estimation of blood loss.

We have used the stapling device for some cesarean sections, but I do not have any data on its use or on the operative times. We keep records of start time and finish time, but these data were not analyzed.

We don't have definitive criteria for blood transfusion. In the past 5 years our tendency to transfuse has decreased. In the early 1980s, anyone with a hematocrit <30% and certainly anyone with associated endometritis received transfusions. Now our criteria are much more strict. A majority of these transfusions were done intraoperatively, when there was uterine atony and a great deal of blood loss.

Amnioinfusion: An aid in the ultrasonographic evaluation of severe oligohydramnios in pregnancy

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OBJECTIVE: Amnioinfusion was used in pregnancy complicated by severe oligohydramnios to enable a more comprehensive ultrasonographic evaluation of the pregnancy.

STUDY DESIGN: Thirteen patients were referred to the Division of Obstetrical and Gynecological Ultrasound at the University of Miami because of severe oligohydramnios in which a definitive diagnosis could not be made. Nine patients were in the second trimester and four were in the early third trimester. All patients underwent amnioinfusion with warm physiologic saline solution and instillation of indigo carmine. Genetic amniocentesis was performed in 11 patients.

RESULTS: After amnioinfusion a satisfactory comprehensive ultrasonographic examination could be performed on all patients. In most cases we were able to achieve a definitive diagnosis.

CONCLUSION: Amnioinfusion with the addition of indigo carmine was a satisfactory technique to enable a more comprehensive ultrasonographic evaluation of pregnancy complicated by severe oligohydramnios.

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Key words: Amnioinfusion, oligohydramnios, ultrasonography

Pregnancy complicated by severe oligohydramnios presents very challenging diagnostic and management problems. The diagnostic considerations include premature rupture of the membranes, genitourinary malformations, other major congenital anomalies, chromosomal abnormalities, intrauterine growth retardation (IUGR), and fetal infections. Ordinarily, targeted high-resolution ultrasonography is very helpful in answering some of these diagnostic dilemmas, often in conjunction with amniocentesis or fetal blood sampling. In the presence of severe oligohydramnios these diagnostic modalities are often inadequate or technically unable to be performed. In 1985 Romero et al.[1] reported both false-positive and false-negative diagnosis of renal agenesis in patients with severe oligohydramnios. Wladimiroff[2] in 1975 reported on the administration of furosemide to mothers with oligohydramnios. Fetal urine production was observed in those with functioning urinary systems. We have not found this

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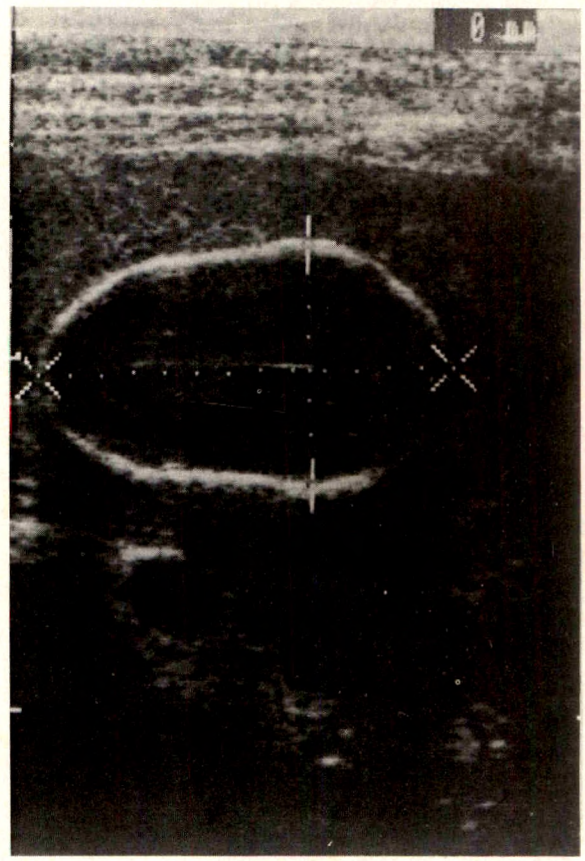


Fig. 1. Preamnioinfusion sonogram of fetal head in case 13.

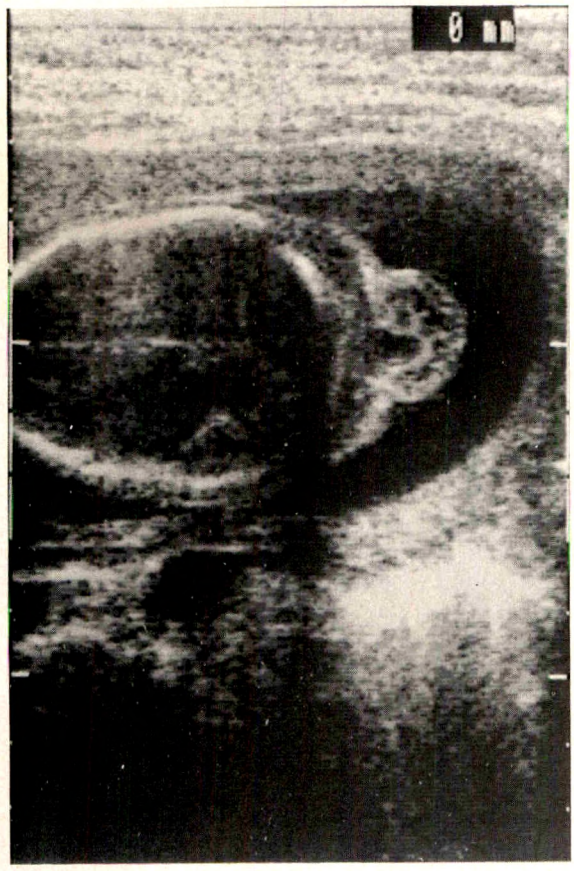


Fig. 2. Postamnioinfusion sonogram revealing occipital encephalocele of fetal head in case 13.

technique to be helpful in our laboratory. Amnioinfusion has been described and is being used in late pregnancy, in the intrapartum period, to prevent meconium aspiration and to eliminate variable or prolonged decelerations.[3-5] In 1988 Gembruch and Hansmann[6] reported on artificial instillation of amniotic fluid to enhance ultrasonographic identification of fetal anatomy in patients with oligohydramnios. In 1989 Nicolini et al.[7] reported two cases of severe oligohydramnios in which fetal intraperitoneal infusion of normal saline solution was used to enhance ultrasonographic evaluation.

We present 13 cases of severe oligohydramnios in which intraamniotic amnioinfusion of normal saline solution was used to enhance the ultrasonographic evaluation of pregnancy.

Material and methods

Thirteen patients were referred to our laboratory with the diagnosis of oligohydramnios so severe that a satisfactory ultrasonographic examination could not be performed. Nine patients were in the second trimester of pregnancy and four were in the third trimester. The gestational age ranged from 14 to 31 weeks. Four patients had elevated maternal serum α-fetoprotein. Two patients were diabetic. None had premature rupture of the membranes documented clinically with certainty.

Because of the severity of the oligohydramnios and the inability to perform an adequate ultrasonographic evaluation, a diagnosis was not possible. The decision was made to perform an intraamniotic infusion with warm (0.9N) physiologic saline solution. Only those patients in which the oligohydramnios was so severe as to make an adequate ultrasonographic examination impossible were subjected to amnioinfusion. A 22-gauge spinal needle was placed in the amniotic sac under direct ultrasonographic guidance. At times an electronic biopsy guide was helpful in placing the needle in a very small pocket. The volume of saline solution infused ranged from 60 to 350 ml. The volume targeted for infusion was 200 ml. This varied, however, on the basis of the volume needed to enable an adequate ultrasonographic examination. The saline solution was infused slowly over a 15- to 20-minute period in 20 ml aliquots with gentle pressure on a 20 ml syringe. To rule in or out premature rupture of the membranes, 3 ml of indigo carmine was instilled, with the same needle, into the amniotic sac after completion of the saline infusion. Because of the association of chromosomal abnormalities with severe oligohydramnios, genetic amniocentesis was performed in 11 of the 13 patients. With the infusion needle still in the amniotic cavity, a small volume of fluid was aspirated and sent for chromosomal analysis.

Results

Thirteen patients with severe oligohydramnios in pregnancy underwent amnioinfusion because of our inability to perform a satisfactory ultrasonographic examination and arrive at a diagnosis to explain the cause or association of the severe oligohydramnios.

Upon infusion of saline solution and a reaccumulation of an amniotic fluid volume, there was a dramatic increase in fetal movement that was not present before the amnioinfusion. Preinfusion ultrasonography (Fig. 1) failed to reveal a major malformation in the fetus of an insulin-dependent diabetic mother at 20 weeks' gestation; the fetus had severe oligohydramnios. The malformation became very apparent (Fig. 2) after the infusion of 220 ml of saline solution.

Of the 13 patients infused (Table I), adequate ultrasonographic evaluation was possible in 12. One patient had gross vaginal leakage of fluid during the infusion, which obviated the amniotic accumulation. With the addition of indigo carmine we could make positive diagnosis of premature rupture of membranes in six patients and eliminate the diagnosis of it in the remaining seven patients. Intrauterine growth retardation of the symmetric type was diagnosed in four of the patients.

Table I. Postamnioinfusion ultrasonographic diagnosis and pregnancy outcome in 13 patients with severe oligohydramnios

Case No.	*Maternal age (yr)*	*Gestational age (wk)*	*Ultrasonographic diagnosis*	*Pregnancy outcome*
1	32	18	Premature rupture of membranes, choroid plexus cyst	Induced abortion
2	28	31	Renal agenesis	Renal agenesis, pulmonary hypoplasia
3	32	21	Intrauterine growth retardation	Delivered at 39 wk, 1640 gm; no anomalies; discharged home
4	33	19	Premature rupture of membranes; no anomalies seen	Induced abortion
5	39	14	Renal agenesis, intrauterine growth retardation	Delivered at 39 wk; neonatal death day 1, 1.4 kg; severe oligohydramnios and Potter's facies; kidneys reported seen at autopsy
6	29	15	Renal agenesis	Chorioamnionitis; spontaneous abortion
7	39	20	Premature rupture of membranes, no anomalies seen	Chorioamnionitis; delivery at 26 wk; discharged home
8	26	23	Renal agenesis, intrauterine growth retardation	Stillborn at 38 wk; no autopsy
9	34	21	Premature rupture of membranes, gross leakage	Chorioamnionitis; spontaneous abortion 21 wk
10	29	25	Premature rupture of membranes, no anomalies seen	Chorioamnionitis; delivery 34 wk; neonatal death day 1
11	26	23	Premature rupture of membranes, no anomalies seen	Chorioamnionitis; delivery 34 wk; discharged home
12	35	20	Intrauterine growth retardation	Delivered at 30 wk, 465 gm; discharged home
13	23	20	Encephalocele, multicystic kidneys	Induced abortion; Meckel-Gruber syndrome confirmed

Table II. Ultrasonographic visualization and diagnostic information after amnioinfusion in 13 patients with severe oligohydramnios

	No.	*%*
Ultrasonographic Visualization		
Improved	12	92.3
Not improved	1	7.7
Premature rupture of membranes		
Diagnosis confirmed	6	46.2
Diagnosis excluded	7	53.8
Intrauterine Growth Retardation	4	30.7
Fetal malformations		
Major	5	38.5
Minor	1	7.7
Karyotyping from aspirated fluid		
Successful	8	61.5
No growth	2	15.4
Unsuccessful attempt	1	7.7
No attempt made	2	15.4
Abnormal karyotype	0	0

Table III. Summary of outcomes in 13 patients after amnioinfusion for severe oligohydramnios

	No.	*%*
Induced abortion	3	23.1
Spontaneous abortion	2	15.4
Chorioamnionitis	5	38.5
Neonatal death	4	30.8
Discharged home	4	30.8
Overall pregnancy loss	9	69.2

Five major congenital malformations were suspected. Of these, four were renal agenesis. One was confirmed by autopsy and two were suspected clinically but autopsies were not performed. The fourth case had kidneys demonstrated at postmortem examination, but there was severe oligohydramnios and the fetus was described as having Potter's facies. One fetus with encephalocele and multicystic kidneys (Meckel-Gruber syndrome) was confirmed at postmortem examination. Chromosomal studies were attempted in 11 of the 13 patients. With postinfusion aspirated fluid, eight karyotypes were obtained; two failed to grow, and one attempt was unsuccessful. Of the two patients who did not have attempts at karyotyping made, one had premature rupture of membranes with gross leakage of fluid as the saline solution was infused and the second patient had a normal karyotype established by genetic amniocentesis performed earlier in the pregnancy for maternal age and elevated maternal serum α-fetoprotein. No abnormal karyotypes were found in our patients. These findings are summarized in Tables II and III.

The overall pregnancy loss was 69.2% in this small group of patients with severe oligohydramnios. Cho-

rioamnionitis developed in five patients (38.5%). In two patients it developed within 3 days after amnioinfusion, and in three it developed more than 4 weeks after amnioinfusion. Four patients with chorioamnionitis had premature rupture of membranes demonstrated by amnioinfusion and indigo carmine. In one patient with chorioamnionitis premature rupture of membranes could not be documented.

Comment

Amnioinfusion was demonstrated to be a technically simple method to enable a comprehensive ultrasonographic examination of the pregnant patient with severe oligohydramnios. By the addition of indigo carmine the diagnosis of premature rupture of membranes could generally be made accurately and quickly by the demonstration of the passage of blue dye. It was also shown that a karyotype could be obtained in a majority of these patients by culturing the aspirated fluid. The incidences of chorioamnionitis and pregnancy loss were high in this group as could be expected in cases of severe oligohydramnios, most occurring in the second trimester of pregnancy.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

Dr. Edgar O. Horger III, Columbia, South Carolina. I have had experience with 11 early second-trimester amnioinfusions, each of which was performed for the strong suspicion of a fetal renal abnormality. Unfortunately, in two of the 11 cases we had rupture of the membranes very shortly after the amnioinfusion. I assume that we put in too much fluid and built up too much intraamniotic pressure. In your study you set out to give 200 ml of fluid but stopped short, giving anywhere between 60 and 350 ml. Did you make any effort to measure intraamniotic pressure along the way? Does this relate to the possibility of rupture of membranes? Did any of your cases have rupture of membranes shortly after you did the amnioinfusion?

Dr. Quetel. First, we did not measure the pressure either before or after the amnioinfusion. It appears that would be something that would not be very difficult to do. Second, we had six patients with premature rupture of membranes. Attempts had been made before the patients being referred to us to try to establish this diagnosis clinically, and it could not be done. With the indigo carmine, one patient, while still on the examining table, had gross leakage of blue fluid into the vagina during the amnioinfusion. Another had the blue dye passed during the first hour after the procedure. It is difficult to say whether we caused the premature rupture of membranes, but we know in our cases of premature rupture of membranes there were no other associated conditions to explain the oligohydramnios.

Dr. Mara J. Dinsmoor, Richmond, Virginia. I was curious about the five patients with chorioamnionitis. Did these patients already have ruptured membranes or did they develop chorioamnionitis after the amnioinfusion?

Dr. Quetel. This has been reported previously by another author. We found that in the six cases of chorioamnionitis five had premature rupture of the membranes and one did not.

Long-term outcome in fetal hydrops from parvovirus B19 infection

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Parvovirus B19 infection in the fetus is associated with anemia and hydrops and can result in fetal death. Fetal transfusion has been used in an attempt to improve outcome; however, it is associated with its own perinatal morbidity. We report two cases of fetal parvovirus B19 infection that were confirmed by polymerase chain reaction for parvovirus B19 deoxyribonucleic acid in umbilical cord blood. Ultrasonographic signs of compromise were observed at 30 and 24 weeks of gestation. Both fetuses were hydropic and one fetus was also anemic. Serial sonograms demonstrated that the hydrops resolved spontaneously over 3 to 5 weeks after diagnosis. One infant was delivered at 32 weeks of gestation as a result of idiopathic preterm labor. The other infant was delivered at term. Both infants appeared relatively normal at birth and have developed normally in the first year of life. Thus fetal hydrops in association with parvovirus B19 infection does not always lead to poor long-term outcome. A conservative approach without in utero therapy may be appropriate for the management of some of these fetuses. (Am J Obstet Gynecol 1992;167:337-41.)

Key words: Parvovirus B19, hydrops, deoxyribonucleic acid polymerase chain reaction

Human parvovirus B19 was identified in 1975[1] and has been associated with in utero fetal death since 1984.[2] Maternal symptoms are variable and do not correlate consistently with the presence or severity of fetal infection. Pregnancies at any gestational age can be affected. Infection may have no effect on the fetus or can result in aplastic anemia, nonimmunologic hydrops, and increased perinatal mortality.[3-5]

Because fetal parvovirus B19 infection can have a variety of outcomes, agreement is lacking on the management. Fetal hydrops is frequently associated with poor prognosis,[6] prompting attempts at invasive in utero treatment that may be appropriate but is not without risks. We will present two cases of in utero parvovirus B19 infection with hydrops resolving without therapeutic intervention.

Case reports

Case 1. A 26-year-old white woman, gravida 2, para 0, aborta 1, was referred at 30 weeks of gestation for fetal hydrops. Her prenatal course had been uncomplicated until the most recent examination revealed that the fundal height exceeded that expected for gestational age. She denied any rash or arthralgia but complained of a flulike illness 3 weeks before her office visit.

Ultrasonographic evaluation revealed ascites, pericardial effusion, abdominal wall edema, bilateral hydroceles, and severe polyhydramnios (Fig. 1). Gestational age by biparietal diameter and femur length was consistent with 30 weeks. No congenital anomalies were identified. The mother was blood type A-positive and had a negative indirect Coombs screen. Acute and convalescent maternal tests for toxoplasmosis, rubella, cytomegalovirus, and herpes (TORCH) titers showed no evidence of a recent infection. There was no evidence of fetomaternal transfusion by the Kleihauer-Betke test. Maternal serum was immunoglobulin (Ig) G positive and IgM negative to parvovirus B19 by enzyme immunoassay. A diagnostic cordocentesis was performed. The fetal complete blood cell count revealed hemoglobin of 14.3 gm/dl, white blood cells 2.3×10^9/L, platelets 288×10^9/L, nucleated red blood cells 8/100 white blood cells, lactate dehydrogenase 179 U/L, aspartate transaminase 19 U/L, alanine aminotransferase 3 U/L, γ-glutamyl transferase 1889 U/L, total protein 3.5 gm/dl, and albumin 2.2 gm/dl. The karyotype was 46,XY. Fetal serum was IgG positive but IgM equivocal to parvovirus B19. Deoxyribonucleic acid (DNA) of parvovirus B19 was detected in the serum by polymerase chain reaction determined by the Centers for Disease Control; however, the results were not available until 8 weeks after the procedure. Amniotic fluid was IgG positive but IgM negative to parvovirus B19 by enzyme immunoassay. The patient was advised that the prognosis was guarded. Although no specific therapy could be recommended, we suggested that she record daily fetal movement, have twice weekly nonstress fetal heart rate testing, and weekly ultrasonographic evaluations to monitor fetal status.

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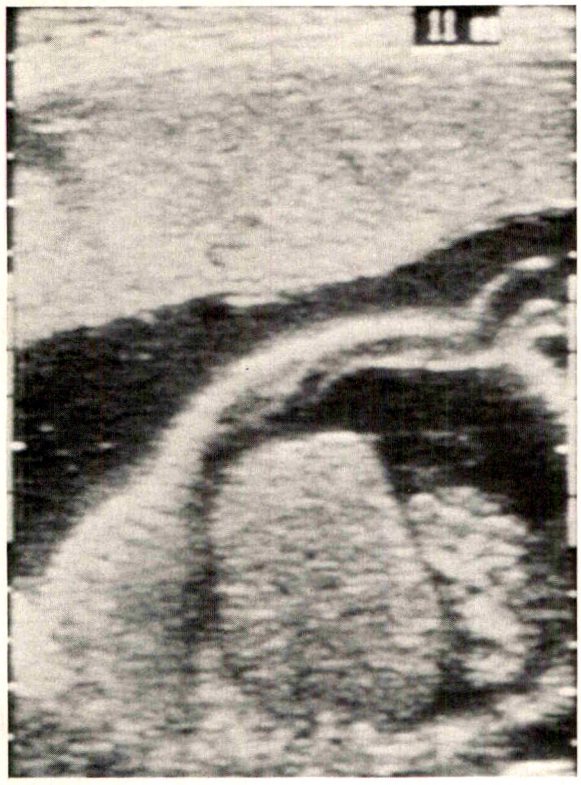


Fig. 1. Severe fetal ascites with bowel, liver, and umbilical vein readily apparent.

The ascites began decreasing by 34 weeks of gestation. By 36 weeks the hydrops had resolved and the amniotic fluid appeared only mildly increased. The amniotic fluid index was normal by 38 weeks.

Labor was induced at 41½ weeks of gestation. The patient required a cesarean section for transverse arrest in the second stage of labor. A 3550 gm male with Apgar scores of 8 and 9 at 1 and 5 minutes, respectively, and a flaccid abdominal wall was delivered. No organomegaly was demonstrated by radiography or ultrasonography. At birth, the hemoglobin was 16.3 gm/dl, white blood cell count 13.5×10^9/L, platelets 332×10^9/L, nucleated red blood cells 1/100 white blood cell count, total protein 6.4 gm/dl, albumin 3.6 gm/dl, and γ-glutamyl transferase 270 U/L.

At 3 months of age the infant was meeting appropriate developmental milestones, However, he was noted to have poor abdominal muscle tone, and both the liver and spleen were felt to be palpable 2 to 3 cm below the costal margin. An ultrasonographic examination did not demonstrate any significant organomegaly. At 9 months of age the baby appears to be normal.

Case 2. A 28-year-old white woman, gravida 4, para 2, abortus 1, was referred at 24 weeks of gestation for fetal hydrops. At 17 weeks of gestation the patient developed a nonpruritic macular rash that began on the trunk and spread to the face and extremities. The rash resolved spontaneously after 5 to 7 days and was followed by symmetric arthralgia involving feet, hands, and knees. The arthralgia also resolved spontaneously. Maternal IgG and IgM for parvovirus B19 obtained during this period were positive. Maternal blood type was O positive and indirect Coombs screen was negative. The maternal serum α-fetoprotein level (AFP) at 16 weeks of gestation was normal.

At 24 weeks an ultrasonographic examination was performed because the fundal height was larger than expected. The fetus had abdominal ascites, pericardial effusion, severe polyhydramnios, and an edematous placenta. Biparietal diameter and femur length were consistent with 23 weeks of gestation. Acute and convalescent TORCH titers first drawn at 23 weeks of gestation did not indicate an acute infection, nor was there evidence of fetomaternal transfusion by the Kleihauer-Betke test.

One week later the sonogram demonstrated a slight improvement of the ascites and polyhydramnios. The pericardial effusion was unchanged, but cardiac contractility appeared normal. Cordocentesis was performed with the intention of transfusing the fetus if it was severely anemic. Amniotic fluid (AFP) was normal (3.33 μg/ml). The karyotype was 46,XX. The fetal hemoglobin was 11.7 gm/dl, and white blood cells 2.6×10^9/L. Fetal serum was IgG positive but IgM equivocal to parvovirus B19 by enzyme immunoassay. The polymerase chain reaction was subsequently reported to be positive for parvovirus B19 DNA in the fetal serum. The patient was counseled that the prognosis was poor for hydrops fetalis resulting from a parvovirus B19 infection. She was offered weekly sonograms in addition to recording daily fetal movement.

By 26 weeks the ascites and polyhydramnios had resolved. The pericardial effusion was undetectable in all subsequent examinations.

The patient went into spontaneous premature labor at 32 weeks of gestation. A 1495 gm female was delivered with Apgar scores of 9 and 9 at 1 and 5 minutes, respectively. Neonatal hemoglobin was 19.8 gm/dl, platelets 119×10^9/L, white blood cells 6.6×10^9/L, and nucleated red blood cells 1/100 white blood cells. Parvovirus B19 DNA was not detected in the neonate's serum obtained shortly after birth. The infant had mild respiratory distress syndrome, but the physical examination was otherwise normal. The infant was discharged 38 days after birth. Placental histologic studies showed no significant infectious changes.

At 18 months of age the child had normal abdominal tone and was meeting the appropriate developmental milestones.

Comment

In these two cases of severe hydrops fetalis resulting from fetal parvovirus B19 infection, the fetal manifestations of infection spontaneously resolved within 5 weeks of diagnosis. This observation suggests an alternate course of management instead of previously described recommendations of in utero fetal therapy.

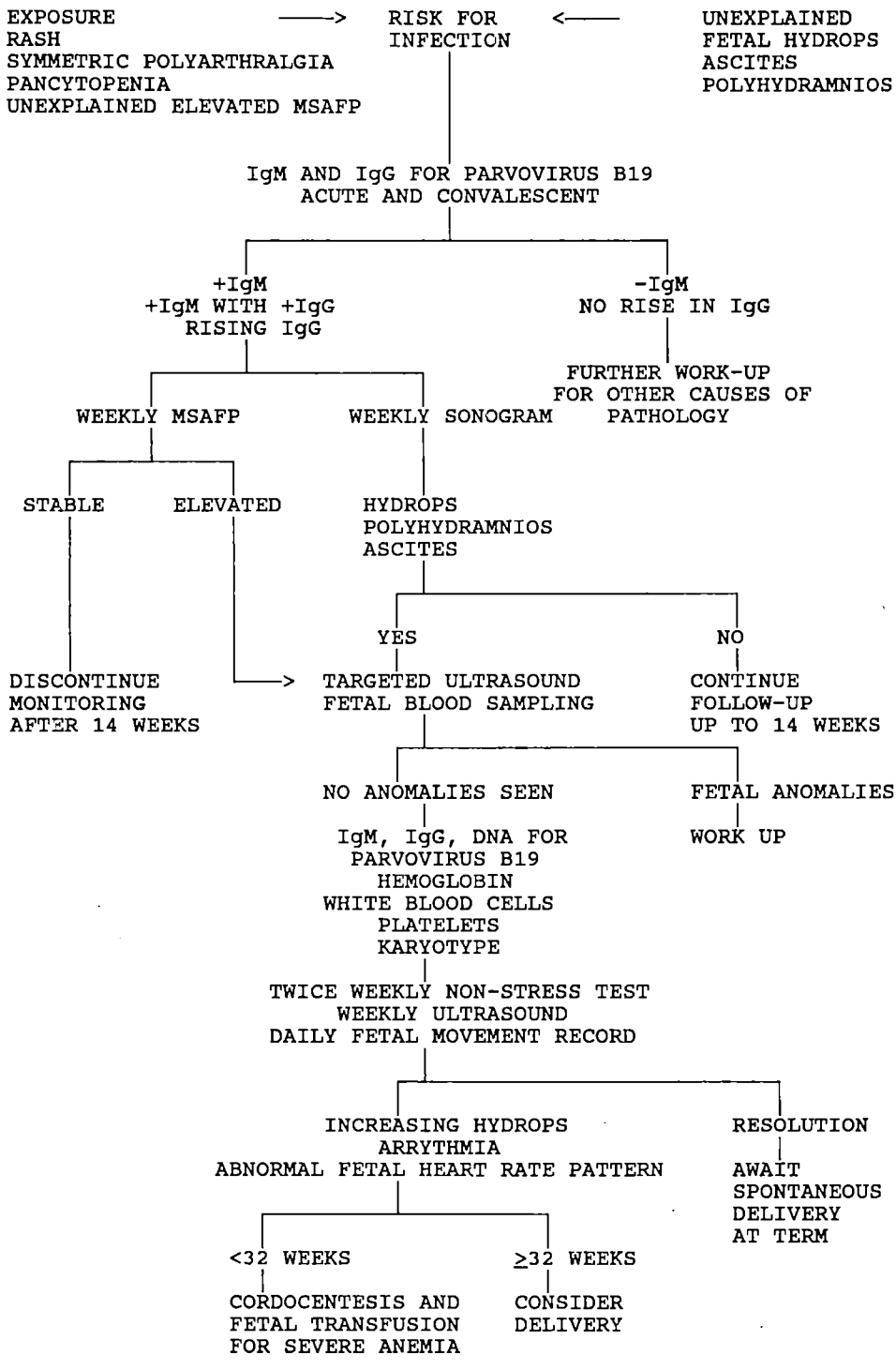


Fig. 2. Flow chart.

Approximately 35% to 50% of the general population is susceptible to parvovirus B19 infection; 20% of this group will become infected after exposure.[7] The risk for infection is highest among household contacts and in working conditions involving close contact, such as in schools.[8] The Centers for Disease Control have estimated that the risk of fetal death from infection on the basis of the risk for maternal infection and the transmission rate to the fetus ranges from <1% to 15%.[9]

Parvovirus B19 infection, or Fifth disease, should be suspected if there has been a local outbreak or if the mother has symmetric polyarthralgia or a maculopapular rash. In children, however, the "slapped cheek"

rash is a more common presentation. Ultrasonographic signs of fetal infection include ascites, effusions, skin edema, polyhydramnios, cardiomegaly, placentomegaly, and decreased fetal movement.[7] Unexplained elevated maternal serum AFP[10] may also indicate infection. The fetus may be anemic or pancytopenic, which can be confirmed by cordocentesis. Diagnosis is confirmed by counterimmunoelectrophoresis or radioimmunoassay for IgM to parvovirus B19 in the maternal serum. Electron microscopy of blood or tissue is useful only when viral particles are seen. DNA tests for parvovirus B19 have become available and are highly sensitive. However, they are only positive in blood during viremia or if the tissue is currently infected.[11, 12]

There are several mechanisms by which the pathologic changes associated with infection occur. Fetal anemia results from arrest of red blood cell maturation[13] and karyorrhexis of red blood cells.[14] Infection of the myocardium may result in direct impairment of cardiac function.[15, 16] Hepatic function may also be impaired directly by infection or by hemosiderin deposition in the hepatocytes and Kupffer cells, which results in necrosis and fibrosis.[17, 18]

Although hydrops is frequently associated with poor prognosis, attempts have been made to use maternal serum AFP or cardiac size to predict outcome. Maternal serum AFP increases 4 weeks before ultrasonographically detectable signs and 6 weeks before fetal death in infected pregnancies.[10] Normal values for maternal serum AFP have been determined through 36 weeks of gestation.[19, 20] Elevation of maternal serum AFP may be the result of hepatic or placental damage. In hydropic fetuses an increased cardiac biventricular outer diameter >95th percentile is predictive of perinatal death.[21]

Past treatments have included in utero fetal transfusion,[22] direct fetal digitalization,[15] and serial thoracentesis or paracentesis.[23] These have been combined with frequent reevaluation by ultrasonography, fetal nonstress tests, or fetal movement records.[7, 15, 23] An alternate approach is outlined in Fig. 2. If infection is suspected, specimens for maternal IgM and IgG titers are drawn immediately and measurements are repeated in 4 weeks. Patients with evidence of previous exposure and no acute rise in IgG require no further evaluation for parvovirus B19 infection. Other causes of hydramnios or hydrops should be sought in those pregnancies where parvovirus B19 infection is not identified. Serial evaluation including weekly maternal serum AFP and sonogram screening should be offered to patients with acute infection as diagnosed by the presence of IgM, IgM, and IgG, DNA, or an acute rise in IgG to parvovirus B19. This weekly screening should be performed for up to 14 weeks after the diagnosis of infection is confirmed.[3, 16]

In the presence of hydrops at or beyond 20 weeks of gestation, diagnostic cordocentesis is recommended. The testing should include karyotype, hemoglobin, platelet count, and white blood cell count in addition to DNA, IgG, and IgM for parvovirus B19. A conservative approach combining twice weekly fetal nonstress tests, serial sonograms, and recording of fetal movement may be used after 28 weeks. Biophysical profiles may not be helpful because anemic fetuses may still have adequate breathing, movement, and tone. Delivery should be considered when the fetus is at or beyond 32 weeks of gestation in cases where hydrops is increasing, arrhythmias are present, or fetal heart rate patterns show no variability or late decelerations. If severe prematurity precludes delivery, cordocentesis for reevaluation of hemoglobin should be performed. If the fetus is severely anemic (for example hemoglobin ≤5 gm/dl), packed red blood cells may be transfused.

A conservative approach without in utero treatment is reasonable in light of several reports that have shown recovery of fetal red cell production.[2, 24] One of our patients had normal hemoglobin and the other was mildly anemic. This is consistent with the recovery phase of parvovirus B19 infection. Spontaneous resolution of fetal parvovirus B19 infection may occur more frequently than previously suspected. Two previous case reports document parvovirus B19 infection with hydrops fetalis and subsequent spontaneous resolution.[23, 25] On the basis of these cases, the short-term prognosis of in utero parvovirus B19 infection resulting in hydrops fetalis may be favorable. Further, the two cases described here suggest that the long-term prognosis after resolution of hydrops is good. Conservative management without in utero fetal therapy is reasonable in selected cases.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

Dr. Edgar O. Horger III, Columbia, South Carolina. Dr. Sheikh, I feel that you and your coauthors showed remarkable restraint in the conservative management of the first case with hydrops at 30 weeks' gestation. I probably would have pushed for delivery, and retrospectively I think that I would have been making a mistake. Let me ask you a question that seems obvious: Can I get parvovirus from my dog?

Dr. Asad U. Sheikh (Closing). Canine parvovirus infection is frequently fatal. However, this organism is not transmitted to humans and does not appear to cause infections in humans.

Experience with mepivacaine paracervical block in an obstetric private practice

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OBJECTIVE: The objective of this study was to determine the efficacy and safety of mepivacaine paracervical block in term, low-risk obstetric patients.
STUDY DESIGN: Over a 3-year period all term, low-risk obstetric patients in the author's private practice were offered mepivacaine paracervical block anesthesia. One hundred eighty-two patients received 200 mg mepivacaine with each block. The patients who declined paracervical block were used as a control group to compare Apgar scores, subject to χ^2 analysis.
RESULTS: Initial injections were 97.3% successful. Average duration of anesthesia was 59.7 minutes with the first injection. Fetal bradycardia occurred in 13.2% with an average duration of 4.5 minutes. The slightly higher incidence of low Apgar scores at 1 and 5 minutes in the study group was not statistically significant. One neonatal death occurred secondary to group B streptococcal sepsis in the study group.
CONCLUSION: Mepivacaine paracervical block remains an anesthetic option in low-risk patients. The high success rate is limited by its short duration of action and the incidence of fetal bradycardia. (AM J OBSTET GYNECOL 1992;167:342-5.)

Key words: Mepivacaine, paracervical block

Paracervical block for obstetric anesthesia was initiated in Germany in the 1920s and became popular in the United States in the mid-1950s.[1] Even with the frequent occurrence of fetal bradycardia, paracervical block was presumed safe until serious maternal and fetal complications were reported in the 1960s. Perinatal deaths were described in both complicated and low-risk labors.[2,3] Since then paracervical block has decreased in popularity, and its use is limited to pregnancies with low risk of underlying fetal compromise. With the current use of electronic fetal monitoring some of the complications reported 30 years ago might be avoided.

The author is a member of an obstetrics-gynecology group practice that offers paracervical block as one of the anesthesia techniques available to laboring patients. It is discussed with the patient by the physicians and by nurse-instructors during childbirth preparation classes. The results of a 3-year experience are presented.

Material and methods

The patients included in this study are all the patients managed by the author between Jan. 1, 1988, and Dec. 31, 1990, who requested and received mepivacaine paracervical block during labor. During this period 197 patients received paracervical blocks; however, lidocaine was used in 15 patients. Therefore 182 patients are reported in this series. The characteristics of this group are recorded in Table I. Any patient who was preterm, after 42 weeks, hypertensive, demonstrating an abnormal fetal heart tone tracing, or exhibiting thick meconium-stained amniotic fluid was not given paracervical block. Patients with light meconium-stained fluid were allowed to receive paracervical blocks. A control group of the remainder of the patients delivered by the author during this 3-year period was used to compare the incidence of low Apgar scores. This control group consisted of 343 patients with characteristics

Table I. Characteristics of 182 patients receiving paracervical block

Characteristic	*No. of patients*
37 to 42 Weeks' gestation	182
Normal fetal heart tracing before block	182
Light meconium	11
Gestational diabetes	2
Vaginal birth after previous cesarean section	2
No prenatal care	1
Premature rupture of membranes <24 hr	1
Von Willebrand's disease	1
Congenital absence of right pulmonary artery (asymptomatic)	1

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6/6/38052

Table II. Efficiency and duration of mepivacaine paracervical block

Paracervical block No.	*Success rate*		*Average duration (min)*
	No.	*%*	
1	177/182	97.3	59.7
2	65/69	94.2	52.1
3	12/12	100	46.7

similar to the study group, but the controls underwent anesthetic techniques other than paracervical block.

The method of injecting the paracervical block involved the use of a disposable paracervical-pudendal block tray that does not allow the needletip more than 5 mm beyond the guide. The patient was placed in lithotomy position. After the vulvar area was washed with a chlorhexidine solution, the guide was placed in one of the lateral fornices of the vagina, aimed toward the pelvic sidewall and away from the fetal head. The needle was inserted through the guide to a depth of ≤5mm. After aspiration to rule out intravenous injection, 10 ml of 1% mepivacaine was injected with aspiration after every 2 to 3 ml. A similar injection was then performed in the other lateral vaginal fornix with no delay other than the time required to resposition the guide and the needle. The patient received a total of 200 mg mepivacaine with each paracervical block.

Results

The anesthetic efficiency of mepivacaine paracervical block in these 182 patients is summarized in Table II. Successful anesthesia was judged only by the patient, so a successful block was noted if the patient stated that she had experienced pain relief. The efficiency of repeat injections is also reported in Table II. Sixty-nine patients required a second block, and 12 needed a third after the anesthetic effects of earlier injections faded.

The duration of initial and repeat paracervical blocks is also recorded in Table II. Only successful blocks were used to determine average duration. The range of duration was 14 to 120 minutes. Duration of <30 minutes was always associated with delivery within 35 minutes of injection. As shown in Table II, duration of effect decreases with subsequent blocks.

Maternal side effects in this group are infrequent. Transient tinnitus or oral paresthesias occurred in only four patients (2.2%). There were no seizures, significant blood pressure changes, or hematomas in these patients.

Fetal bradycardia was the most common side effect. It was defined as a fetal heart rate drop below 100 beats/min (or 40 beats/min below previous baseline) lasting more than 30 seconds and occurring within 45 minutes of paracervical block. Early decelerations associated with pushing were not included. Incidence, injection to bradycardia interval, and duration of bradycardia are reported in Table III. Management of this complication included increasing maternal intravenous fluid infusion, maternal oxygen therapy by mask, and maternal positioning on the left side. All episodes of bradycardia responded without any further intervention. The range of duration of fetal bradycardia was 1 to 8 minutes.

Table III. Bradycardia with mepivacaine paracervical block in 182 patients

Incidence	*Average injection-bradycardia interval (min)*	*Average duration (min)*
24/182 (13.2%)	7.9	4.5

Table IV. Incidence of Apgar scores <7

Group	*1 min Apgar score <7*	*5 min Apgar score <7*
Paracervical block	19/182 (10.4%)	4/182 (2.2%)
Control	24/343 (7.0%)	4/343 (1.2%)

Data were evaluated by χ^2 statistical analysis. Differences in 1-minute Apgar scores between the paracervical block group and control group ($\chi^2 = 1.87$) and differences in 5-minute Apgar scores between the groups ($\chi^2 = 0.84$) were not statistically significant.

A comparison of low Apgar scores (<7) between the paracervical block group and the control group is presented in Table IV. There is a higher incidence of low Apgar scores at 1 and 5 minutes in the paracervical block group, but the differences are small. Of the 19 study patients with 1-minute Apgar scores <7, seven were associated with shoulder dystocia, five had experienced fetal bradycardia, three had meconium, two had a tight nuchal cord, and one had group B streptococcal infection. Of the four babies with 5-minute Apgar scores <7, two were associated with shoulder dystocia, one with tight nuchal cord, one with light meconium, and none had fetal bradycardia from paracervical block.

There was one neonatal death in the paracervical block group, but this was an effect of group B streptococcal infection rather than an anesthetic complication. The patient was a 34-year-old woman, gravida 6, para 2-0-3-2, with a benign prenatal course. At 37 weeks she was in labor, and the cervix was 3 cm dilated and 80% effaced with the vertex at −2 station and with intact membranes. Amniotomy was performed, and clear fluid was noted. She received 50 mg meperidine hydrochloride intravenously, and 2 hours later, at 7 cm dilatation, she requested paracervical block. Relief was obtained for 68 minutes; at 75 minutes after the first

paracervical block a second injection was performed at 8 cm dilatation. Outlet vacuum extraction delivery of a 2750 gm male infant occurred 31 minutes after the second block. There was no fetal bradycardia, maternal fever, or meconium. Apgar scores were 6 at 1 minute and 7 at 5 minutes. The baby was transported from the birthing room to the nursery with oxygen. The initial working diagnosis was transient tachypnea versus sepsis. Sepsis evaluation was performed and chest radiography showed pneumonia. In spite of aggressive antibiotic, respiratory, and fluid management, the condition of the infant deteriorated and he died 14 hours after birth. Blood cultures grew group B streptococci.

Comment

The success rate for mepivacaine paracervical block in this group of 182 patients was excellent. However, duration of action of only 1 hour with the first block and even less with subsequent injections requires frequent repeat procedures. This exposes the patient to higher total doses of local anesthetics with greater potential for toxicity. The 13.2% incidence of bradycardia was comparable to earlier studies. The occurrence of lower Apgar scores was also slightly higher in the paracervical group. These findings reinforce the prudence of using this anesthetic technique in low-risk patients only. Perhaps the ideal patient is one who is multiparous, at term with no evidence of fetal compromise, and experiencing a rapid labor.

The worst fetal outcome during this 3-year experience was a neonatal death associated with group B streptococcal infection. This outcome was unrelated to the patient's paracervical block.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

DR. ALLAN G. MCLEOD, Miami, Florida. In 1992 a report of 182 patients receiving mepivacaine paracervical block for labor analgesia is unusual and of special interest to me in that I have used the technique extensively in the past. In the late 1960s and 1970s, as stated by Dr. Goins, the technique engendered a reputation for serious complications, resulting in diminished popularity of the technique, especially in the hostile medicolegal climate. Second, its duration of action was relatively short, and obstetric anesthesiology services, including epidural anesthesia, were becoming increasingly available to obstetricians as the subspecialty of obstetric anesthesiology began to gain a foothold.

Patients in this study were carefully selected to exclude parturients at increased risk.

The paracervical block technique was carefully described by Dr. Goins correctly emphasizing preinjection aspiration, depth of penetration, and correct positioning of the needle to avoid intravascular injection or injection directly into the fetal scalp. These are important factors in reducing the complication rate. Personally, I tended to delay the injection in to the second fornix for a brief interval while monitoring the fetus; this appeared to minimize the incidence of bradycardia. In more advanced labor, placing the examining fingers between the fetal head and the needle insertion point in the fornix is an extra safeguard. I commend Dr. Goins also for the careful vulvar cleansing performed before the injection but had some concern regarding use of the lithotomy position for any prolonged period.

An Apgar score <7 at 5 minutes did not appear to be a complication of the block itself in Dr. Goins' study. The only death in the series was related to a β-streptococcal infection.

Dr. Goins, while reporting a high success rate, emphasizes such drawbacks as short duration of action and the need for repeat injection with resultant exposure to higher total doses of local anesthetic agents. I concur with his statement that the normal multiparous woman requiring analgesia is probably the ideal candidate for this technique. It would have been interesting to learn the extent of the discussion of the technique with couples at the childbirth preparation classes.

Fetal bradycardia occurred in 13.2% of cases in this series, which is less than the 25% to 35% reported by others. This may well be attributable to the careful technique of Dr. Goins. The injection-to-bradycardia interval averaged 7.9 minutes with an average duration of 4.5 minutes. Shnider et al.[1] noted an onset within 2 to 10 minutes and a duration of 3 to 30 minutes and emphasized that whereas the cause of the bradycardia was controversial its significance was not. Increased neonatal morbidity and >50 cases of perinatal death associated with paracervical block have been reported in the American and European literature. Shnider et al. emphasized that paracervical block should not be used in situations where the fetus is potentially or actually compromised.

Dr. Goins injected 10 ml mepivacaine on each side. I found it possible to obtain a satisfactory block with 5 to 7 ml on each side. In addition to the safeguards already mentioned, the drug dosage should be kept to a minimum.

Bupivacaine should not be used for paracervical block. Freeman and Arnold[2] suggested that chloroprocaine should be used rather than mepivacaine or lidocaine if more than one dose was likely to be required.

Dr. Goins has demonstrated that paracervical block can be used safely in an obstetric practice; however, use

of the technique demands that the user and the parturient are fully aware of the hazards associated with its use and the steps necessary to minimize their occurrence.

Most obstetric services have discontinued the use of paracervical block in favor of epidural anesthesia or other forms of analgesia, and use of the technique tends to be limited to those units with minimal obstetric anesthesiology services or small community hospitals.

I should like to congratulate Dr. Goins on his study and caution him to be ever vigilant in his selection of patients and in applying the appropriate safeguards in the use of the technique and, finally, to recognize clearly that he is "swimming against the tide" in the unfortunate event that he should experience a serious complication in the future.

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DR. GOINS (Closing). As far as counseling patients is concerned, I explained how I perform the technique and that our incidence of bradycardia is about 15%. The patients are also told that should bradycardia occur, they will be placed on a side and given oxygen. They understand that in the past the bradycardia has responded to this. It is important that they know ahead of time about the possibility of bradycardia, because they are very aware of changes in the monitor, almost staring at the monitor like children in front of a television. The dosage that I currently use is based on past experience. Lower dosages failed to give an anesthetic effect of sufficient duration. The use of paracervical anesthesia is limited to low-risk patients.

I appreciate the discussion of the cause of the bradycardia that is associated with paracervical block, which I did not touch on in my article.

Dr. Hopwood's article is one that I reviewed. In the group of patients with 5-minute Apgar scores <7 none of the patients experienced fetal bradycardia. Of the 19 patients who had 1-minute Apgar scores <7, five experienced fetal bradycardia. I do not have it broken down as to the time between injection of the local anesthetic and delivery. Among the patients with low Apgar scores who were delivered within 30 minutes of the block, there was only one baby with a low Apgar score. That baby who subsequently had group B streptococci had a 1-minute Apgar score of 6 and a 5-minute Apgar score of 7.

With regard to the question about scalp pH and cord gas studies, I did not determine scalp pH in any of the patients with fetal bradycardia, and no cord gas studies were done on either the study patients or the control patients.

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Refining the biophysical profile with a risk-related evaluation of test performance

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OBJECTIVE: Our objective was to determine the ability of biophysical profile variables to predict bad perinatal outcome in high-risk third-trimester pregnancy.

STUDY DESIGN: The outcomes of 1146 fetuses were correlated with abnormal single or multiple variables occurring in biophysical profile done within 72 hours of delivery. Theoretic risks of poor outcomes for different combinations of abnormal variables, calculated with Shortliffe's formula, were compared with the actual risks observed (χ^2 tests).

RESULTS: Two hundred forty-six fetuses had at least one abnormal biophysical profile variable with the risk of bad outcome, for a single abnormal variable, ranging from 8% (body movements) to 100% (tone) and increasing from 14% (any variable abnormal) to 63% (all variables abnormal). In most (57%) observed combinations of biophysical profile variables, significant differences between theoretic and actual risks of bad outcomes were found. By stepwise logistic regression the best predictive model contained all variables except fetal movement.

CONCLUSIONS: Dynamic biophysical profile variables appear to be interdependent. Not all combinations of abnormal variables occur and specific combinations improve prediction of poor outcome. Risk-related scales for biophysical profile outcomes might prove superior to more conventional scoring systems. (Am J Obstet Gynecol 1992;167:346-52.)

Key words: High-risk pregnancy, biophysical testing, fetal heart rate, fetal movement, fetal breathing, amniotic fluid volume

The fetal biophysical profile has become an increasingly popular test over the past decade.[1-3] The biophysical profile combines variables reflecting fetal status, e.g., baseline heart rate, breathing movements, and body movements, with those more suggestive of chronic status, e.g., amniotic fluid volume and neurologic tone. This testing approach assumes that the consideration of multiple variables improves diagnostic accuracy and provides a more comprehensive evaluation of fetal condition. Although previous reports have addressed these issues,[4-9] they have shared the following features: (1) test variables have not been collected simultaneously, (2) an arbitrary rating scheme for variables has been used, (3) the length of observation has been relatively short, often ≤30 minutes, (4) standardization of testing conditions has not been strictly enforced, and (5) the prevalence of poor outcomes has been relatively low. Earlier studies have suggested, on the one hand, that there is a progressive sequence in the appearance of abnormal variables,[2] and, on the one hand, that there is little prognostic difference among tests grouped by number of abnormal variables.[1-3] More recently, Manning et al.[10] have questioned the relationship of specific abnormal variables and their combinations to test accuracy. They suggest that there are prognostic differences associated with different combinations of variables and that the unequal distribution of these combinations implies an interdependence of these variables.

We undertook the present study to test the validity of these prior assumptions. First, we wished to determine if there were differences in the prognostic values of individual variables and their combinations for predicting the risk of abnormal outcomes. Next, we wished to determine if, within each grouping of abnormal variables, all combinations of abnormal variables were equally probable. Finally, we wished to determine if a model that assumed independence of variables would yield predictive values that agreed with those actually observed in the field.

Material and methods

The study population consisted of 1146 patients who received antenatal testing at the Medical College of Georgia Hospitals and Clinics for a variety of high-risk problems (Table I). All were tested between 32 and 42

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weeks of gestation and delivered within 72 hours of their final test. Testing results were available to clinicians who provided obstetric care. Although antepartum management was based on a variety of clinical considerations, in general fetuses with normal biophysical profiles were retested within 7 days, those with one abnormal variable were retested within 24 to 48 hours, and those with two or more abnormal variables were evaluated for delivery.

Biophysical testing was performed in accordance with a previously published protocol.[11] Ultrasonographic observations of fetal breathing movements, body movements, amniotic fluid volume, and tone (flexion-extension movements) were acquired simultaneously with a continuous recording of fetal heart rate. These data were entered in a specially programmed microcomputer that analyzed the incidences of fetal breathing movement, body movement, flexion-extensions, accelerations and decelerations, and the baseline fetal heart rate.[11] Amniotic fluid volume was measured as the largest single pocket in vertical and horizontal dimensions. Previously published institutional standards[3] for gestational age were used as reference points. Abnormal values for any variable were considered to be those that exceeded the normal population means by >2 SD.

For the purposes of this study, only the last tests before delivery were considered in the subsequent correlations with outcomes. Perinatal end points examined were mortality, intrapartum fetal distress requiring operative intervention, 5-minute Apgar score <7, neonatal mixed or metabolic acidosis (on the basis of umbilical arterial pH <7.15 and base excess >−8.0 mEq/L). The conditional probability for the risk of bad outcomes associated with each variable was determined by its occurrence as the sole abnormal variable with all bad outcomes.[12] The theoretic risk of bad outcomes associated with combinations of more than one abnormal variable was determined by the following formula of Shortliffe and Buchanan[13]

$$BO = \sum_{a} Ra - \sum_{\substack{a=b \\ a<b}} Ra\,Rb + \sum_{\substack{a=b=c \\ a<b<c}} Ra\,Rb\,Rc \ldots$$

where BO is risk of bad outcome and Ra, Rb, Rc . . . is risk of bad outcome associated with abnormal variable (if the variable is normal, R = O).

The theoretic risks of bad outcomes and the observed rates of bad outcomes associated with each combination of abnormal biophysical profile variables were compared. For the purposes of this study, a level of disagreement >0.2 (range 0 to 1.0) was considered clinically significant. In addition, the distributions of combinations of abnormal variables and their associated positive predictive values were compared with χ^2 tests.

Table I. Indications for biophysical testing

Indications	*No.*	*%*
Postdatism	110	9.6
Suspected intrauterine growth retardation	207	18.1
Chronic hypertension	196	17.1
Preeclampsia	75	6.5
Diabetes mellitus	181	15.3
Poor obstetric history	44	3.8
Miscellaneous obstetric	274	24.4
Miscellaneous medical	54	4.7
TOTAL	1146	100.0

The standard for comparison within each category of abnormal variable combinations, ie, two abnormal variables, three abnormal variables . . . , was the mean positive predictive value for the entire category[10]; no combination with fewer than five cases was considered for this comparison.

Linear logistic regressions were applied to combinations of abnormal variables. A p value <0.05 was considered significant.

Results

The study population was delivered at a gestational age of 38.1 ± 2.1 (mean ± SD) weeks. The distribution of test outcomes by number of abnormal variables and associated perinatal outcomes is listed in Table II. Two hundred forty-six patients (21.5%) had at least one abnormal variable on their last biophysical profile. There was a positive correlation ($r = 0.31, p = 0.04$) between increasing numbers of abnormal variables and the occurrence of all bad outcomes. Similar trends were seen with all individual bad outcomes except for low 5-minute Apgar scores.

Table III shows that the nonreactive nonstress test (NST) was the most common abnormal variable, whereas absent tone was the least common abnormal variable. No individual variable had high sensitivity, whereas all had high specificity. Similarly, positive predictive values for all variables except fetal tone were <50%; all variables had negative predictive values >88%.

Table IV shows the frequency of tests with a single abnormal variable and associated poor outcomes. Although a nonreactive NST occurred significantly more often than the other abnormal variables, absent fetal tone was the only variable uniformly associated with abnormal outcomes. In both cases a major fetal anomaly was present (anencephaly, holoprosencephaly), and neonatal death occurred.

Combinations of two or more abnormal variables and their associated poor perinatal outcomes are shown in Tables V through VII. Six of 10 possible combinations of two abnormal variables were observed (Table V), of

Table II. Distribution of abnormal outcomes and last biophysical profile

Abnormal variables	*Total No.*	*Abnormal outcomes (%)**				
		All	*Intrapartum fetal death†*	*Low 5 min Apgar score*	*Acidosis*	*Death*
0	900	2.7	0.2	7.0	3.6	0.1
1	133	15.0	10.9	4.3	3.7	2.6
2	59	29.0	21.5	7.2	5.2	3.5
3	22	36.0	38.0	11.2	7.4	5.0
4	16	43.0	56.3	31.2	25.0	18.2
5	16	62.5	66.7	62.5	62.5	25.0
TOTAL	1146	7.4	12.3	5.9	5.9	2.9

*Expressed as percentages of all outcomes within variable category.

†Expressed as percentage of cases undergoing spontaneous or induced labor.

Table III. Individual biophysical profile variables and perinatal outcomes (%)*

Variable	*Abnormal*	*Sensitivity*	*Specificity*	*Positive predictive value*	*Negative predictive value*
NST	15	30	88	28	89
Fetal breathing movement	10	28	92	36	89
Body movement	6	21	96	48	89
Amniotic fluid volume	5	13	97	39	88
Tone	4	18	98	60	88

*All tests with single and combined abnormal variables included.

Table IV. Abnormal individual biophysical variables: Frequency and associated perinatal outcomes

Variable	*Frequency*		*Abnormal outcomes*	
	No.	*%*	*No.*	*%*
NST	69	51.9	8	11.6
Fetal breathing movement	34	25.6	6	17.7
Amniotic fluid volume	16	12.0	3	18.8
Body movement	12	9.0	1	8.3
Tone	2	1.5	2	100.0
All tests	133	100.0	20	15.0

which abnormal fetal breathing movement and nonreactive NST were the most frequent. Three of these six combinations of abnormal variables were of sufficient size to allow statistical comparisons. Only the combination of abnormal NST and amniotic fluid volume had a predictive accuracy that was significantly greater than the group mean and approximately twice that calculated for independent variables. Five of 10 possible combinations of three abnormal variables were observed (Table VI), of which abnormal NST–body movement–fetal breathing movement was the most frequent. Only two combinations occurred frequently enough to allow statistical comparisons of predictive accuracy; neither differed significantly from the group mean. The predictive accuracy of the combination of abnormal NST–body movement–fetal tone differed significantly from that predicted for independent variables. Two of five possible combinations of four abnormal variables was observed (Table VII). The predictive accuracy of each combination differed significantly from that predicted for independent variables.

The predictive accuracy of five abnormal variables was 62.5%, although the formula would have yielded a 100% risk of poor outcomes. Had intrauterine growth retardation been considered as a measure of poor outcome, the predictive accuracy would have been 100% because all cases without other complications had birth weights <10th percentile for gestational age.

Sixteen perinatal deaths (three stillbirths and 13 neonatal deaths) occurred in the study group. Only one death followed a normal biophysical profile and was due to complications of prematurity. Of the three neonatal deaths associated with a single abnormal biophysical profile variable, two were in infants with major central nervous system malformations; the other death was a consequence of necrotizing enterocolitis in an otherwise normal term infant. Two stillbirths occurred in the group with two abnormal variables: one in a poorly controlled diabetic patient and the other in a fetus with hydrops caused by congenital heart block.

Table V. Combinations of abnormal variables: Frequency and associated perinatal outcomes observed and predicted

Variable	Frequency		Abnormal outcomes	
	No	*%*	*Observed (%)*	*Predicted (%)*
NST–fetal breathing movement	32	54.1	28.1	27.3
NST–body movement	10	17.0	20.0	19.1
NST–amniotic fluid volume	7	11.9	57.1	28.4
Fetal breathing movement–tone	4	6.8	25.0	59.0
NST–tone	3	5.1	0.0	56.0
Fetal breathing movement–amniotic fluid volume	3	5.1	0.0	33.3
All tests	59	100.0	17 (29.0)	

Table VI. Combinations of three abnormal variables: Frequency and associated perinatal outcomes observed and predicted

Variable	Frequency		Abnormal outcomes	
	No.	*%*	*Observed (%)*	*Predicted (%)*
NST–body movement–fetal breathing movement	8	36.4	37.5	38.0
NST–body movement–tone	6	27.3	33.3	70.0
NST–body movement–amniotic fluid volume	3	13.6	33.3	39.0
NST–fetal breathing movement–amniotic fluid volume	3	13.6	33.3	49.0
NST–fetal breathing movement–tone	2	9.1	50.0	79.0
All tests	22	100.0	8 (36.4)	

The remaining deaths associated with three or more abnormal variables were either consequences of major anomalies (two cases) or intrauterine growth retardation (eight cases).

Logistic regression analysis was applied to all test outcomes and yielded a model that was highly significant for all abnormal outcomes ($\chi^2 = 35.62$, $p < 0.00001$). This model required NST, fetal breathing movement, amniotic fluid volume, and tone for any abnormal outcome, with the highest weighting for tone (2.78) and the lowest for amniotic fluid volume (2.08). Body movements were not required for the prediction of any poor outcome.

Comment

Fetal biophysical testing has entered its second decade of clinical investigation and application. The consensus of previous studies is that the combined observation of several biophysical variables yields a test whose predictive accuracy exceeds that of the NST alone.[14, 15] The extensive data base compiled by Manning et al.[5] suggests that predictive accuracy of poor perinatal outcomes increases significantly as the number of abnormal biophysical variables increases. Vintzileos et al.[16] and Manning et al.[10] have implied that consideration of specific test variables, when abnormal, may be more important for prognosis than a raw score derived from combining unweighted variables. It has been implied further that a modified biophysical profile, focusing primarily on the NST and amniotic fluid volume evaluation, might be sufficient for most antepartum testing needs.[17] These issues are clearly important to resolve because biophysical testing is more labor intensive than the NST or amniotic fluid volume measurement.

The current study has focused on the frequency of associated abnormal biophysical profile variables and their relationship to the risk of a bad perinatal outcome. As in virtually all prior reports of biophysical testing, we have observed a positive relationship between the number of abnormal biophysical profile variables and the risk of bad perinatal outcomes. In contrast to some of the previous studies[3, 5, 6] we also found that tests with only one abnormal variable could be associated with an increased risk of perinatal compromise when compared with tests in which all variables were normal. The most significant abnormal variable, when seen in isolation, appears to be fetal tone, because this abnormality was rare but was always associated with central nervous system anomalies.

Our data tend to support some of the conclusions reached by Manning et al.[10] in a similar previous study. The frequencies of abnormal individual variables either in isolation or in combination with other abnormal variables are not equal. The most common abnormal vari-

Table VII. Combinations of four abnormal variables: Frequency and associated perinatal outcomes observed and predicted

Variable	Frequency		Abnormal outcomes	
	No.	*%*	*Observed (%)*	*Predicted (%)*
All except amniotic fluid volume	11	68.8	31.2	88.0
All except tone	5	31.2	40.0	67.0
All tests	16	100.0	7 (43.7)	

able was the nonreactive NST. This finding agrees with that of Baskett et al.[4] but differs from our previous report[3] and that of Manning et al.[10] in which fetal breathing movement and fetal tone were the most common abnormal variables, respectively. These differences may reflect sample size, gestational age of the population studied, study technique, and cutoff points used for abnormal variables. No individual variable confers sufficient predictive accuracy to be a good "stand-alone" test.

The frequency distribution of the abnormal individual variables is also of interest, because it bears on the "ontogeny" of fetal response to intrauterine compromise proposed by Vintzileos et al.[2, 8] Their model suggests an inverse relationship between the age of appearance and the central nervous systems centers in which biophysical activities develop and their sensitivity to hypoxia. This model implies that hypoxia would affect the newer centers such as those governing fetal heart rate and fetal breathing movement before those regulating body movements and tone. Although our data do not directly confirm a sequential pattern of variable abnormality before poor outcome, the relative frequency of abnormal variables follows the sequence proposed by Vintzileos et al.[2] with nonreactive NSTs being the most common and absence of tone the least common.

This study differs from most of its predecessors in the manner with which the biophysical data were collected and in the standards used for their testing. In our laboratory, both ultrasonographic and fetal heart rate data are collected simultaneously under rigorously standardized environmental conditions. They are rated according to standards that we have established for normal fetuses of similar gestational age.[3] These normative standards are more stringent for all biophysical variables than those used in the other approaches. Although this makes the analysis of the combined abnormal variables not directly comparable to those of Manning et al.,[10] it also avoids possible artifacts that occur when fetal state changes during the course of a study. The failure to observe all possible combinations of abnormal variables may be due partly to sample size but may also reflect their dependent relationship to fetal state. This latter contention is supported by the fact that in no combinations of two or three abnormal variables was decreased body movement seen without abnormal NST, and in all combinations of four abnormal variables, abnormal NST, fetal breathing movement, and body movement occurred together.

A further argument for the interdependence of the biophysical variables is supported by the discrepancies in predicted and observed risks of bad perinatal outcomes in more than half (4/7) of the test combinations sufficiently large to analyze. In most instances (3/4), the observed incidence of poor outcome was considerably lower than that predicted by a formula that assumed that the variables were independent. Our data also support the view that certain combinations of abnormal variables may have greater prognostic importance than others, e.g., the combination of nonreactive NST and abnormal amniotic fluid volume. More important, predictive accuracy for all observed combinations of three or four abnormal variables appears to be similar within their respective categories.

This study adds further support to the original thesis of Manning et al.[1] that multiple variable biophysical testing can augment prediction of outcome in high risk pregnancy. Additional variables improve the predictive accuracy of the biophysical profile; however, they do not appear to be of equal importance for establishing prognosis. The logistic regression model suggests that fetal movement data could be eliminated without reducing predictive accuracy, provided that all variables are collected simultaneously. This is most likely due to its being redundant when collected simultaneously with the NST. Conversely, fetal tone appears to be an essential component of the biophysical profile; its elimination from the logistic model effected the greatest alteration in predictive accuracy.

For practical considerations, this study has shown that when biophysical profiles contain one or two abnormal variables the nature of the specific abnormal variable becomes quite important as the risk of poor outcome shows the greatest variation in these cases. Once three or four variables are abnormal, the specific nature of the variables included in these combinations becomes less important, because predictive accuracy is quite similar for all observed combinations within these groupings. It is also clear that relatively high predictive accuracy is achieved only when all variables are abnormal. This suggests that clinical management after the

majority of abnormal biophysical profiles should rely very strongly on additional clinical data, especially fetal maturity, before intervention is undertaken.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

DR. WILLIAM F. O'BRIEN, South Florida. The manuscript details an analysis of the components of the fetal biophysical profile with respect to outcome in an attempt to establish a system for weighing or condensation of components. The authors used a data base constructed from computer-aided biophysical profiles with institutionally derived criteria obtained on over 1000 women, with only the last test being used for analysis.

The "outcome" used was a group of potential markers of fetal compromise ranging in severity from mortality to a 5-minute Apgar score <7. Analysis essentially compared the actual rate of bad outcomes, which occurred with a combination of abnormal biophysical profile components with the theoretic expectation of risk based on the observed risk when only a single component was abnormal. Finally, the components were entered into a logistic regression model.

As expected from prior reports, the incidence of abnormal fetal outcomes in general increased along with the number of abnormal biophysical profile components. The NST and fetal breathing movements were the components most often found to be abnormal either singly or in combination with other biophysical profile components.

When two component combinations were tested, the combination of abnormal NST and amniotic fluid volume components appeared to be particularly ominous, with an observed rate of bad outcomes almost twice that predicted. This result was almost certainly due to the fallacies inherent in dealing with limited numbers, because this combination had better results when either the fetal motion or fetal breathing movement component was abnormal. Most of the obvious discrepancies were related to the fetal tone component, as a result of the overprediction of risk on the basis of abnormal fetal tone. This was a single abnormal component only twice and in both cases a major central nervous system anomaly resulted in neonatal death.

These results conflict with those reported by Manning et al.[1] in a similar study. In that study the combination of abnormal NST and body movement, which was the second most common combination of two abnormalities noted in this manuscript, was not recorded in over 500 tests. Second, they found the combination of abnormal NST and fetal breathing movement to yield significantly greater morbidity than other combinations of two abnormal values, whereas as noted above Devoe et al. did not.

With regard to logistic regression, both reports agree on three of the components (fetal breathing movement, NST, amniotic fluid volume), but Manning et al. did not include fetal tone, as does the present report. Weighing of the variables is also different, moreover, because the highest weight is given to tone in this report.

The reasons for these differences are not certain, but several possibilities exist. As in all comparisons, the populations studied were not identical and, in this case, they were not even similar. The mean gestational age at delivery in the study by Manning et al. was 31 weeks, compared with 38 weeks in this study. Perhaps the relative importance of the components of the biophysical profile is gestational-age related. Alternatively, the criteria used for normalcy for the components of the bio-

physical profile are similar but not identical. As previously reported by this group, the results in terms of prediction accuracy do differ and the relative importance of the varying components may as well.

What remains, therefore, is the implied, but unstated and unfulfilled, goal of the study. Can a weighted biophysical profile yield more accurate predictions in a population not used to derive the model? I assume this question will be the basis of Dr. Devoe's next study.

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DR. RONALD V. WADE, Charlotte, North Carolina. Dr. Devoe, in view of the data you have presented, would you comment on the common practice of eliminating the NST if all other parameters are normal?

DR. DEVOE (Closing). Our experience and that reported by the Manning group[1] would suggest that elimination of the NST is unwarranted. The reason is that the NST supplies information that is not really replicated by any of the other components of the biophysical test. In addition, Manning's own experience in eliminating the NST resulted in reports of stillbirths with otherwise normal biophysical profiles within a week of their last test.[1] In cases in which the NST was included, in his particular series, the rate of stillbirths was extremely low unless anomalies were detected. I think that our data support the importance of the NST as a pivotal member of all the variables grouped together in the biophysical profile. It certainly weighed very heavily in all of the abnormal test combinations and was the most frequently observed single abnormal variable. Therefore our data would support maintaining the NST as an integral component of the biophysical profile.

I would like to turn to the comments that Dr. O'Brien made because I think that he has brought out a couple of significant points. First and foremost is the influence of gestational age on the outcome of testing and the outcome of pregnancy in general. There is little question that our study differs significantly from that reported by the Manning group[2] in terms of gestational age at birth and that a number of criteria used to grade morbidity in his population almost certainly reflect the impact of delivery at a lower gestational age in a high-risk population. A second major difference that exists between our study and that previously cited is the fact that we performed the collection of all of our variables in the same time frame. Manning's approach, which has been used by many others, has been to do sequential NST or ultrasonographic evaluation so that it is quite conceivable, in that time frame, that the fetal state would change and a discrepancy between the association of variables could be accounted for.

I think that the challenge that Dr. O'Brien has put forth of doing a prospective study is certainly one that is important and it is in progress at our institution.

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Excessive maternal weight and pregnancy outcome

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OBJECTIVES: This study was undertaken to determine the influences of increased maternal prepregnancy weight and increased gestational weight gain on pregnancy outcome.
STUDY DESIGN: This was a longitudinal retrospective study of 7407 term pregnancies delivered from 1987 through 1989. After excluding cases with multiple fetuses, stillbirths, fetal anomalies, no prenatal care, selected medical and surgical complications, and those with incomplete medical records, 3191 cases remained for analyses by determination of odds ratios for obstetric outcomes, by χ^2 tests for significant differences and by adjustment for risk factors with stepwise logistic regression.
RESULTS: Both increased maternal prepregnancy weight (body mass index) and increased maternal gestational weight gain were associated with increased risks of fetal macrosomia ($p < 0.0001$), labor abnormalities ($p < 0.0001$), postdatism ($p = 0.002$), meconium staining ($p < 0.001$), and unscheduled cesarean sections ($p < 0.0001$). They were also associated with decreased frequencies of low birth weight ($p < 0.001$). The magnitude of the last was less than that of the other outcomes.
CONCLUSIONS: Increased maternal weight gain in pregnancy results in higher frequencies of fetal macrosomia, which in turn lead to increased rates of cesarean section and other major maternal and fetal complications. Because these costs of increased maternal weight gain appear to outweigh benefits, weight gain recommendations for pregnancy warrant careful review. (Am J Obstet Gynecol 1992;167:353-72.)

Key words: Pregnancy, body mass index, weight gain, cesarean section

In recent years there has been much interest in the association between poor maternal weight gain and low birth weight (LBW).[1-4] Because of these observations, recommendations have recently been made by the National Academy of Science to advise further weight gain increases during pregnancy.[5] These recommendations have been made on the basis of several assumptions: (1) The morbidity and mortality associated with LBW is due in part to poor maternal nutrition, as reflected by low gestational weight gain. (2) Through nutritional counseling and support, maternal gestational weight gain can be increased. (3) Increasing gestational weight gain will eliminate or reduce the frequency of LBW complications.

Unfortunately, these assumptions have not been evaluated by carefully designed, recent studies in a United States sample. However, on the basis of these assumptions there have been frequent articles in the lay press encouraging pregnant patients to eat "to appetite."[6, 7] Publications dealing with prepregnancy counseling and preparation for pregnancy give limited attention to the potential complications of excessive maternal prepregnancy weight and excessive gain during pregnancy.

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6/6/38060

These considerations are of great interest to clinicians, because of an increasing percentage of obesity among pregnant patients[5] and because of the greater cesarean section and complication rates observed among such patients. Accordingly, this study was undertaken to assess the adverse effects of excessive maternal weight on pregnancy outcome. There have been a number of other studies addressing these issues.[8-13] This analysis is different in that it focuses on a recently delivered population of women, the majority of whom received nutrition counseling and the benefits of modern obstetric, anesthetic, and pediatric care. It therefore reflects current improvements in care. It also explores the influence of prepregnancy status (body mass index) as defined and categorized in the recent National Academy of Sciences publication[5] and weight gain during pregnancy, in conjunction with other important risk factors.

Methods

With a computerized data system for all deliveries at our institution from Jan. 1, 1987, through Dec. 31, 1989, we identified a total of 7407 patients delivered at or beyond 38 weeks of gestation. The study group consisted predominantly of patients who received care from the prenatal clinics of the University of Florida, Departments of Obstetrics and Gynecology. These clinics serve a twelve-county section of North Central Florida that includes a college town but is otherwise predominantly rural. Three to four percent were private patients. The majority of the remaining patients were

of low-income status (≤150% of federal poverty level). All patients were counseled by a nutritionist and encouraged to gain ≥24 pounds. No limit was placed on maximum weight gain. Glucose screening was routinely done for diabetes mellitus on all patients at 24 to 28 weeks' gestation.

Information on each patient was entered on standardized forms at the first antepartal visit, at delivery, and at discharge from the hospital. After verification for accuracy, the nearly 200 variables were computerized. All singleton pregnancies delivered at ≥38 weeks that received prenatal care and were delivered of live infants in Shands Hospital were included in this study, provided they were not diagnosed with fetal abnormalities, oligohydramnios, or polyhydramnios. There were 6634 patients meeting these criteria. Another 1463 patients with identified relevant medical or surgical complications (gastrointestinal disorders, sickle cell hemoglobinopathy, hepatitis, hematologic disorders, malignant disease, renal disease, neurologic disease, pulmonary disease, psychiatric disorders, tuberculosis) were excluded. Patients with appendicitis, nonhemoglobinopathic anemia (hematocrit <30%), bone or joint disease, urinary tract infections, and asthma were retained because their exclusion would have significantly reduced the total numbers and because these complications did not appear to influence the outcomes of interest. For purposes of this study, we grouped all patients together who voluntarily admitted to tobacco, alcohol, or illicit drug use as patients admitting to substance abuse. Patients with the diagnoses of diabetes, preeclampsia, and chronic hypertension were included for special studies as noted. Seven hundred four cases were excluded because of incomplete risk variable data; an additional 1276 cases were excluded because of incomplete outcome variable information. The final number of deliveries left for analyses was 3191. The mean sociodemographic characteristics (maternal age, parity, level of education, ethnic group proportions, percentage receiving private medical care, number of prenatal visits, and gestational age) and the mean anthropometric features (maternal height, body mass index, gestational weight gain, net weight gain, and infant birth weights) for the entire sample were not significantly different from the study sample ($p > 0.05$). Pregravid weight was determined on the basis of maternal reporting. The weight recorded at the last prenatal visit was designated as the maternal weight at delivery. The mean length of time between last prenatal visit and delivery was 6.1 ± 6 days. Weight gain during pregnancy was calculated by subtracting the self-reported prepregnancy weight from the maternal weight last recorded before delivery. Height was measured and recorded on registration. Birth weight was measured and recorded at delivery.

Gestational age was assigned by the physician delivering the patient on the basis of the best estimate from menstrual data, physical examination, and ultrasonographic studies. Race, marital status, private or nonprivate care, educational level, and parity were all obtained from the prenatal history. Information concerning drug abuse was voluntarily provided by the patients during routine questioning, usually on the first prenatal visit. Intrapartum complications were recorded by the physicians delivering the patients, including the presence or absence of fetal heart rate (FHR) abnormalities, labor abnormalities, and meconium staining. Fetal gender, birth weight, Apgar scores, and the presence or absence of neonatal resuscitation were derived from delivery room data.

We chose to evaluate the relationships between certain outcomes and the following risk factors: (1) prepregnancy weight stratified into quartiles; (2) height (<62 inches, 62 to 65 inches, and >65 inches); (3) body mass index stratified into the four groupings recommended by the National Academy of Sciences[5]; (4) maternal gestational weight gain stratified into quartiles for this patient population, into quartiles of net gestational weight gain (total maternal weight gain minus baby's birth weight), or according to National Academy of Science recommendations for body mass index, as being below, within, or above the recommended range[5] (patients with obese body mass indexes were assigned a recommended range of 15 to 25 pounds); (5) ethnic groups (white, black, and other); (6) gestational age 38 to 42 and >42 weeks; (7) married or single; (8) private physician care or not; (9) education (less than high school graduate, high school graduate, and any beyond high school graduation); (10) parous or not; (11) maternal age (<20, 20 to 26, and >26 years); (12) admitted smoking, alcohol, or illicit drug use; (13) fetal gender; (14) birth weight categorized into <2500 gm, 2500 to 4000 gm, and ≥4000 gm; (15) maternal diabetes (pregestational and gestational) or not; and (16) hypertensive or not.

Outcome variables studied included (1) fetal macrosomia (≥4000 gm) and (2) low birth weight (<2500 gm) where the risk factor birth weight (<2500 gm, 2500 to 4000 gm, and >4000 gm) was excluded. The risk factor of birth weight was included in assessing the following outcomes: (3) meconium staining, (4) 1-minute Apgar scores, (5) 5-minute Apgar scores, (6) unscheduled cesarean sections, (7) oxytocin induction, (8) oxytocin augmentation, (9) labor abnormality (prolonged latent phase, protracted active phase, secondary arrest of dilation, arrest of descent, prolonged second stage), (10) FHR abnormality (decreased variability, bradycardia, or tachycardia for >10 minutes, multiple variables, or late decelerations), (11) cord umbilical artery pH <7.12, (12) newborn resuscitation (artificial ventilation

Table I. Study population characteristics by body mass index group

Characteristic	Body mass index category				
	Low (<19.8) (n = 755)	*Medium (19.8-26) (n = 1621)*	*High (27-29) (n = 329)*	*Obese (>29) (n = 486)*	*All (n = 3191)*
Prepregnancy weight quartile (lb)					
1 (≤116)	582	228	0	0	810
2 (117-132)	161	644	5	0	810
3 (133-155)	12	654	105	24	795
4 (>155)	0	95	219	462	776
Height (in)					
1 (<62)	89	220	54	88	451
2 (62-65)	363	859	160	257	1639
3 (>65)	303	542	115	141	1101
Weight gain (lb)					
1 (<16)	59	190	65	157	471
2 (16-25)	140	292	63	107	602
3 (26-35)	265	467	93	84	909
4 (>35)	291	672	108	138	1209
Net weight gain (lb)					
1 (<14.9)	133	341	101	221	796
2 (14.9-23.5)	207	409	80	102	798
3 (24-33.0)	229	404	85	83	801
4 (>33.0)	186	467	63	80	796
Weight gain, National Academy of Science					
Low	254	431	49	137	871
Within range	317	518	79	127	1041
Over	184	672	201	222	1279
Ethnic group					
White	487	972	164	252	1875
Black	254	615	161	231	1261
Other	14	34	4	3	55
Private physician					
No	734	1555	317	476	3082
Yes	21	66	12	10	109
Married					
No	433	874	196	246	1749
Yes	322	747	133	240	1442
Tobacco, alcohol, drugs					
No	455	1042	230	341	2068
Yes	300	579	99	145	1123
Parity					
First	372	698	123	151	1344
Multiparous	383	923	206	335	1847
Male infant					
No	381	812	166	239	1598
Yes	374	809	163	247	1593
Maternal age (yr)					
<20	276	500	85	80	941
20-26	338	754	161	262	1515
>26	141	367	83	144	735
Maternal education					
<12th grade	390	729	135	177	1431
12th grade	285	676	156	237	1354
>12th grade	80	216	38	72	406
Hypertension					
Yes	26	74	19	52	171
No	729	1547	310	434	3020
Diabetes					
Yes	14	38	20	26	98
No	741	1583	309	460	3093
Birth weight (gm)					
<2500	36	32	5	1	74
2500-4000	673	1381	273	401	2728
>4000	46	208	51	84	389
Gestational age ≥42 wk					
No	694	1476	289	430	2877
Yes	61	157	40	56	314

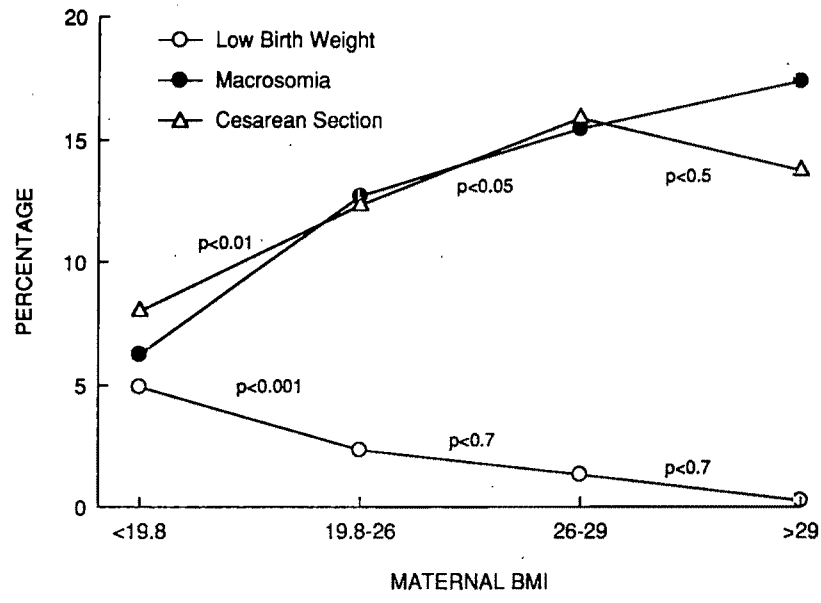


Fig. 1. This graph of unadjusted data shows the relationship between increasing maternal body mass index *(BMI)* and frequencies of macrosomia, cesarean section, and LBW. There is a very similar rise in both macrosomia and cesarean section rates with increasing maternal body mass index. *p* Values represent statistical significance between successive rates of cesarean section. Decrease in percentage of LBW (of lesser magnitude) is also noted with increasing maternal body mass index.

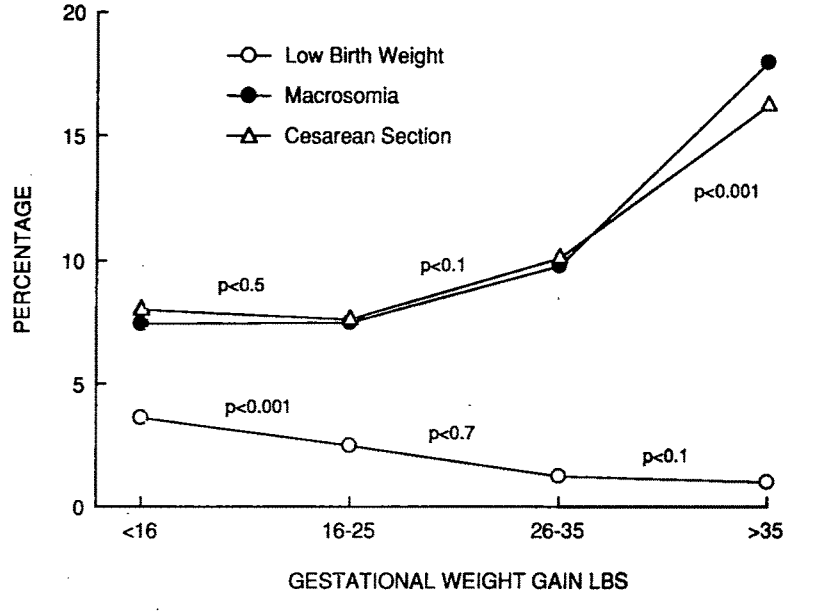


Fig. 2. This graph illustrates unadjusted relationships between increasing maternal gestational weight gain and frequencies of macrosomia, cesarean section, and LBW. As in Fig. 1, there is almost identical alteration in frequencies of macrosomia and cesarean section with increasing gestational weight gain. *p* Values represent statistical significance for differences between successive rates of cesarean section. There is corresponding decrease in LBW of lesser magnitude, also noted with rising gestational weight gain.

and endotracheal intubation), (13) shoulder dystocia, and (14) postdates (gestational age excluded as a risk factor). Patients with the diagnosis of preeclampsia (including pregnancy-induced hypertension, preeclampsia mild or severe, and eclampsia) and chronic hypertension (with or without superimposed preeclampsia) were included in assessing the associations between risk factors and the occurrence of these specific complications.

For the statistical evaluations, all risk factors were

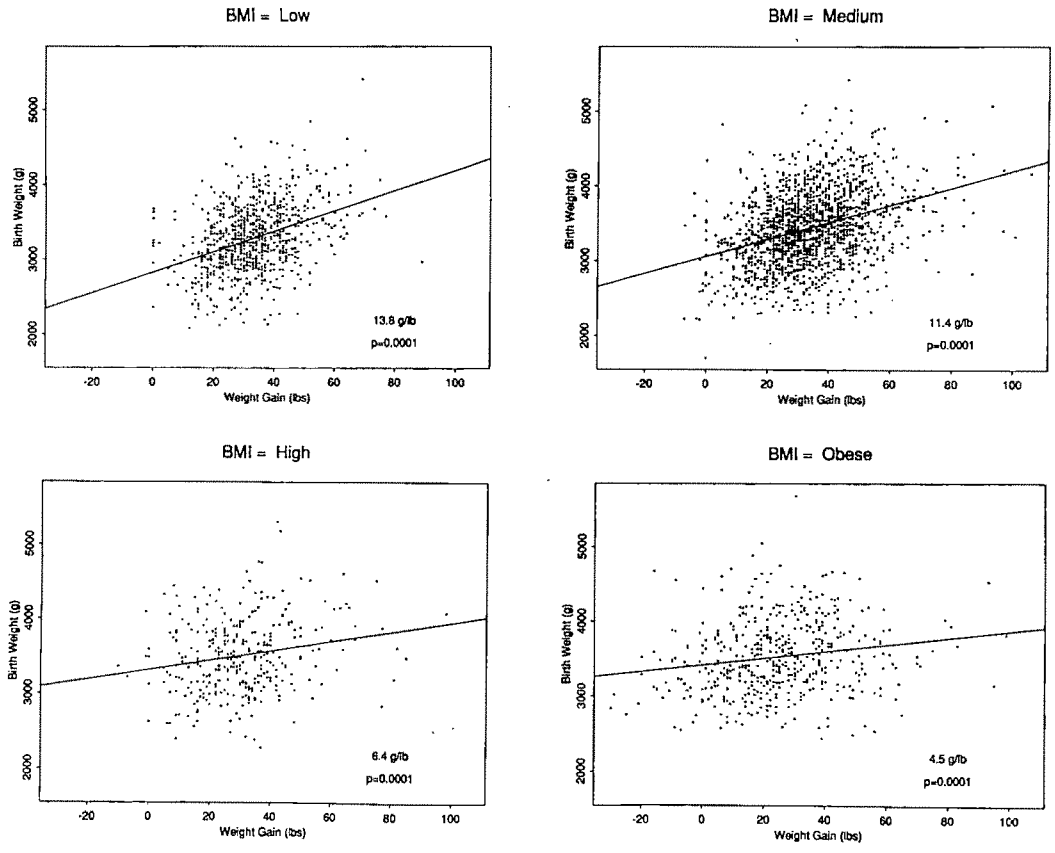


Fig. 3. This scattergram graph depicts unadjusted relationship between weight gain and birth weight in the four body mass index *(BMI)* categories. Substantial variation in birth weight is readily seen. There is significant reduction in association of weight gain to birth weight as body mass index increases, but association even among obese patients is statistically significant ($p < 0.0001$).

divided into intervals as described above, with odds ratios used to describe the effect of each level of a risk factor, relative to a reference group. The significance of each unadjusted odds ratio was assessed with a χ^2 test. Stepwise logistic regression was used to select an adjustment model, and the adjusted odds ratios were derived from the anti-logs of parameters estimated by logistic regression. The Wald χ^2 statistic provided a significance level for each adjusted odds ratio, and confidence intervals for odds ratios were formed by transforming the estimates, give or take two standard errors, from the log odds scale. Weight gain, prepregnancy weight, and height were included in each adjustment model, regardless of statistical significance.

In the trend analyses all risk factors were examined, but only those selected by a stepwise procedure at the 0.01 level of significance were included in the model. Net weight gain was used when examining birth weight and the incidences of macrosomia and low birth weight. The magnitudes of the trends in risk of an outcome were expressed as odds ratio for either a 10 pound difference in weight gain, or a five unit difference in body mass index, because these were round numbers near the average width of the intervals used in the National Academy of Science categories and recommendations.

This research project was approved by the University of Florida Health Center Institutional Review Board.

Results

The characteristics of our study population are tabulated in Table I. There were 3191 total patients in the study, including 171 with the diagnosis of hypertension (including preeclampsia) and 98 with the diagnosis of diabetes. According to body mass index category, 24% were below normal, 51% were within normal range, 10% were above average, and 15% were obese. About 58% of the patients were white, 40% were black, and 1.7% were Hispanic or Oriental. Overall, 55% were unmarried and 42% were nulliparous. High school graduation or education beyond high school may be used as a measure of moderate to high socioeconomic status. About 55% achieved these levels of education. Private physician care was used by 3.7% of all patients. The prevalence of admitted substance abuse (smoking, alcohol, or illicit drugs) was 35%.

Table II. Fetal macrosomia

Characteristic	*%*	*Odds ratio and 95% confidence interval*	*p Value*	*Adjusted odds ratio and 95% confidence interval*	*p Value*
Prepregnancy weight quartile (lb)					
1* (≤116)	5.8	—	—	—	—
2 (117-132)	11.7	2.16 (1.5-3.1)	0.0001	NS	—
3 (133-155)	11.8	2.18 (1.5-3.1)	0.0001	NS	—
4 (>155)	19.7	3.99 (2.8-5.6)	0.0001	3.55 (2.64-4.78)	0.0001
Height (in)					
1* (<62)	5.5	—	—	—	—
2 (62-65)	12.1	2.3 (1.5-3.6)	0.0001	1.9 (1.2-3.1)	0.53
3 (>65)	15.1	3.0 (1.9-4.7)	0.0001	2.2 (1.3-3.6)	0.11
Body mass index category, National Academy of Science					
Low* (<19.8)	6.1	—	—	—	—
Medium (19.8-26)	12.8	2.27 (1.6-3.2)	0.0001	1.82 (1.38-2.4)	0.0001
High (27-29)	15.5	2.83 (1.8-4.3)	0.0001	—	0.44
Obese (>29)	17.3	3.22 (2.2-4.7)	0.0001	—	0.97
Net weight gain (lb)					
1* (14.9)	8.5	—	—	—	—
2 (14.9-23.5)	8.9	1.05 (0.74-1.48)	0.8	1.2 (0.83-1.75)	0.32
3 (24-33)	12.6	1.54 (1.12-2.14)	0.008	1.77 (1.24-2.52)	0.0012
4 (>33)	18.7	2.46 (1.8-3.3)	0.0001	2.86 (2.02-4.02)	0.0001
Ethnic group					
White*	15.5	—	—	—	—
Black	7.4	0.43 (0.34-0.55)	0.0001	0.36 (0.27-0.47)	0.0001
Other	9.1	0.54 (0.22-1.38)	0.19	NS	—
Gestational age ≥42 wk					
No*	11.2	—	—	—	—
Yes	21.0	2.1 (1.6-2.8)	0.0001	1.9 (1.4-2.6)	0.0001
Married					
No*	11.2	—	—	—	—
Yes	15.6	1.8 (1.4-2.2)	0.0001	—	0.68
Tobacco, alcohol, drugs					
No*	13.9	—	—	—	—
Yes	9.0	0.61 (0.48-0.78)	0.0001	0.5 (0.39-0.66)	0.0001
First birth					
Yes*	9.7	—	—	—	—
No	14.0	1.5 (1.2-1.88)	0.0001	1.75 (1.37-2.24)	0.0001
Fetal sex male					
No*	9.3	—	—	—	—
Yes	15.1	1.7 (1.4-2.1)	0.0001	1.76 (1.4-2.23)	0.0001
Maternal age (yr)					
<20	8.8	0.66 (0.5-0.87)	0.003	—	0.48
20-26*	12.7	—	—	—	—
>26	15.4	1.24 (0.97-1.6)	0.087	—	0.75
Education					
<12th Grade	10.6	0.89 (0.7-1.12)	0.318	—	0.88
12th Grade*	11.8	—	—	—	—
>12th Grade	19.0	1.75 (1.29-2.35)	0.0001	1.6 (1.18-2.16)	0.0018
Diabetes					
No*	11.9	—	—	—	—
Yes	22.4	2.15 (1.32-3.5)	0.002	1.75 (1.03-2.97)	0.035

NS, Not significant. Overall incidence 12.2%.

*Reference group.

In this population the frequencies of fetal macrosomia and unscheduled cesarean section appear to rise almost simultaneously in relationship to increasing maternal body mass index (Fig. 1) and to increasing gestational weight gain (Fig. 2).

As both body mass index and gestational weight gain rise, the incidences of macrosomia and cesarean section increase to involve one of six patients (8.9% to 14% or 15%, $p < 0.001$), whereas the LBW rate declines (4% to 1%, $p < 0.001$).

Birth weight. The unadjusted data displayed a strong association between gestational weight gain and birth weight within the four body mass index groups (Fig. 3). This association was less among women with higher body mass index but still very significant ($p = 0.0001$). A multiple regression of birth weight on net weight gain, body mass index, and seven covariables selected by stepwise fitting revealed an increase of 15.4 ± 2.2 gm birth weight per pound gained by the mother ($p < 0.0001$) and an increase of 66.9 ± 95 gm

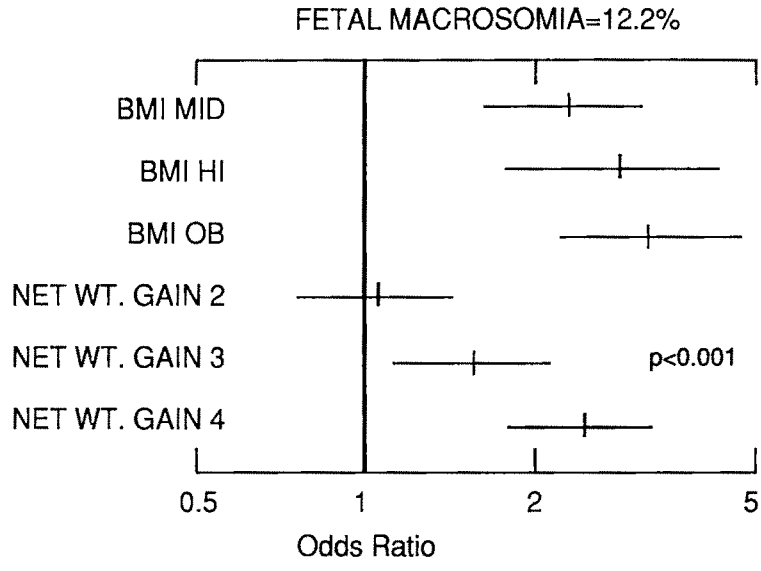


Fig. 4. This graph depicts stepwise increase in unadjusted odds ratios for fetal macrosomia with increasing maternal body mass index and increasing net gestational weight gain. Adjusted trends test was significant at $p < 0.001$. *BMI*, Body mass index; *OB*, obese.

per unit body mass index ($p < 0.0001$). Height, race (black vs white), postdate delivery, substance abuse, fetal gender, parity, and diabetes were all associated with birth weight change ($p < 0.0006$). The effect of body mass index appeared to be nonlinear, leveling off at higher values, but no curvature was detectable in the association with weight gain, adjusting for covariables. Age, education, marital status, private physician, and hypertension did not appear related to birth weight ($p \geq 0.01$).

Macrosomia and low birth weight. The overall incidence of fetal macrosomia, defined as a birth weight of ≥4000 gm, was 12.2% for this study population (Table II). The frequency of macrosomia increased in association with increasing prepregnancy weight, maternal height, maternal body mass index, and net gestational weight gain. Both body mass index and net weight gain demonstrated significant odds ratios for macrosomia (Fig. 4).

After adjusting for risk factors by stepwise logistic regression, the model still exhibited significantly increased ratios for the highest prepregnancy weight group, the medium body mass index group, and increasing net weight gain. The lack of a statistically significant adjusted odds ratio for high or obese body mass index categories may be due to the smaller numbers in these categories and associations with confounding risk factors. The adjusted odds ratios also were significantly elevated for postdatism, parous patients, patients with higher education, those delivering male infants, and diabetics. Patients admitting to substance abuse and black patients were at reduced risk. After adjusting maternal height and age, marital status, and hypertension did not exhibit significantly altered odds ratios for macrosomia (Fig. 5).

The trends analysis done by stepwise logistic regression revealed increasing risk of macrosomia with both increasing weight gain (odds ratio 1.3 for a 10-pound difference, $p = 0.0001$) and with increasing body mass index (odds ratio 1.5 for a 5-unit difference, $p = 0.0001$), adjusting for height, race, education, substance abuse, fetal gender, parity, and postdate delivery. (No interactions or nonlinearity involving weight gain and body mass index were detected.)

The frequency of diagnosed shoulder dystocia was 1.8%. There was a trend to increasing odds ratio noted with increasing maternal gestational weight gain, but after adjusting for the listed risk factors only macrosomia was significantly associated with an increased odds ratio: 5.3 (3.0-9.3; $p < 0.0001$) compared with infants weighing 2500 to 4000 gm.

Defining LBW as <2500 gm, we noted an overall incidence of 2.3% in this term population (Table III). Maternal prepregnancy weight, maternal body mass index, and net gestational weight gain related inversely to the risk of LBW (Fig. 6). Male fetuses had a significantly lower odds ratio for LBW. Patients admitting to substance abuse had a higher risk of LBW. After adjusting for these risk factors, high maternal prepregnancy weight, higher net weight gain, multiparity, and delivering a male infant were associated with low odds ratios for LBW. Patients admitting to drug abuse and black patients demonstrated increased odds ratios.

The trends analysis of LBW demonstrated an estimated odds ratio of 1.4 ($p = 0.0007$) associated with a

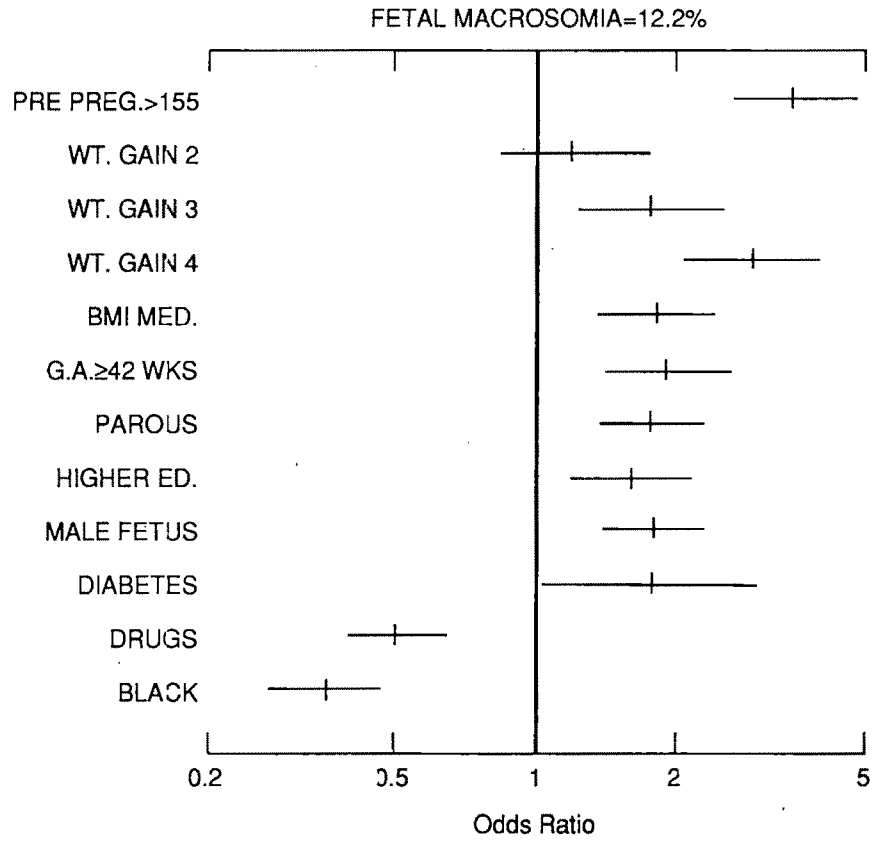


Fig. 5. This graph illustrates odds ratios and 95% confidence intervals for fetal macrosomia for variety of risk factors, after adjustment by stepwise logistic regression. Number of obstetric and demographic characteristics were associated with increased fetal macrosomia. Black patients and patients admitting to substance abuse (drugs) were noted to have significantly reduced frequencies of fetal macrosomia. *BMI*, Body mass index.

10-pound difference in weight gain, and an odds ratio of almost 3 ($p = 0.0001$) associated with a 5-unit difference in body mass index.

Unscheduled cesarean section. In analyzing the frequency of cesarean section, we chose to exclude all electively scheduled cesarean sections and analyzed only those occurring because of complications during labor. The overall incidence for this study population was 11.9% (Table IV). A significant increase in the odds ratio for cesarean section was noted with the higher body mass index categories, the higher prepregnancy weight categories, and the greater gestational weight gain categories. Macrosomia, nulliparity, higher education, diabetes, hypertension, and private care were also associated with increased odds ratios for cesarean section. Adjusting for risk factors yielded significantly increased odds ratios for the highest gestational weight gain group, private care, nulliparity, and fetal macrosomia. Being a teenager and being tall were both associated with reduced odds ratios for unscheduled cesarean section (Fig. 7). Maternal race, substance abuse, and fetal gender did not appear to influence this rate. The trends analysis done by stepwise logistic regression demonstrated an increased risk of unscheduled cesarean section associated with increasing birth weight ($p < 0.0001$), maternal gestational weight gain ($p < 0.0001$), and body mass index ($p < 0.0001$).

Postdates. The overall frequency for postdate pregnancies in this study population was 9.8% (Table V). Increased odds ratios were noted with higher maternal prepregnancy weight, higher gestational weight gain, white patients, fetal macrosomia, and admitted substance abuse. After adjusting for these various risk factors, the odds ratios were significantly elevated only for higher prepregnancy weight, patients admitting to substance abuse, and patients with fetal macrosomia. Older patients and black patients had reduced odds ratios. There was evidence on trends analysis that risk increased with weight gain (odds ratio 1.13 for a 10-lb difference, $p = 0.0014$) and with body mass index (odds ratio 1.2 for a 5-unit difference, $p = 0.0003$).

Labor abnormalities. The overall frequency of labor

Table III. LBW

Characteristic	*%*	*Odds ratio and 95% confidence interval*	*p Value*	*Adjusted odds ratio and 95% confidence interval*	*p Value*
Prepregnancy weight quartile (lb)					
1* (≤116)	4.8	—	—	—	—
2 (117-132)	2.8	0.58 (0.34-0.98)	0.038	—	NS
3 (133-155)	1.0	0.20 (0.09-0.4)	0.0001	0.24 (0.11-0.53)	0.0002
4 (>155)	0.5	0.10 (0.04-0.29)	0.0001	0.12 (0.04-0.34)	0.0001
Height (in)					
1 (<62)	4.2	—	—	—	—
2 (62-65)	2.5	0.58 (0.34-1.02)	0.054	—	NS
3 (>65)	1.3	0.29 (0.15-0.59)	0.0001	—	NS
Body mass index category, National Academy of Science					
Low* (<19.8)	4.8	—	—	—	—
Medium (19.8-26)	2.0	0.40 (0.25-0.65)	0.0001	—	0.049
High (27-29)	1.5	0.31 (0.12-0.79)	0.0001	—	0.056
Obese (>29)	0.2	0.04 (0.006-0.30)	0.0001	—	0.10
Net weight gain (lb)					
1* (<14.9)	3.4	—	—	—	—
2 (14.9-23.5)	2.1	0.62 (0.34-1.15)	0.124	0.51 (0.27-0.98)	0.039
3 (24-33)	2.1	0.62 (0.33-1.14)	0.121	0.54 (0.28-1.04)	0.059
4 (>33)	1.6	0.47 (0.24-0.92)	0.025	0.38 (0.2-0.8)	0.007
Ethnic group					
White*	2.1	—	—	—	—
Black	2.6	1.23 (0.8-1.97)	0.378	2.13 (1.25-3.63)	0.004
Other	1.8	0.85 (0.12-0.85)	0.87	—	0.79
Married					
No*	2.7	—	—	—	—
Yes	1.9	0.69 (0.43-1.12)	0.128	—	0.09
Tobacco, alcohol, drugs					
No*	1.4	—	—	—	—
Yes	4.0	2.94 (1.83-4.7)	0.0001	3.9 (2.3-6.7)	0.0001
Parity					
1*	3.1	—	—	—	—
>1	1.8	0.58 (0.36-0.92)	0.019	0.51 (0.31-0.83)	0.006
Fetal sex male					
No*	3.3	—	—	—	—
Yes	1.3	0.39 (0.23-0.65)	0.0001	0.37 (0.22-0.63)	0.0002

NS, Not significant. Overall incidence 2.3%.
*Reference group.

abnormalities was 7.8% (Table VI). There was a significant increase in odds ratio for labor abnormalities noted with the larger body mass index categories, the greatest gestational weight gain group, private patients, nulliparous patients, patients delivering male infants, patients with hypertension, and fetal macrosomia. After adjusting for these multiple factors, labor abnormalities had significantly higher odds ratios in private patients, blacks, nulliparous patients, and patients with a macrosomic fetus but reduced odds ratio in the tallest patient group. The risk of labor abnormalities did not seem to be significantly influenced by maternal age, education attainment, admitted substance abuse, pregnancy duration, nor marital status. There were significant trends in both weight gain and body mass index. A difference in weight gain of 10 pounds corresponds to an odds ratio of 2 ($p < 0.0001$) and a difference of 5 body mass index units corresponded to an odds ratio of about 1.65 ($p < 0.0001$). Labor abnormality was cited as the most frequent indication for unscheduled cesarean section and accounted for 40% of these operations in this study.

The overall incidence of oxytocin induction was 13.7%. There was a significant twofold increase in the odds ratio for induction observed in the highest body mass index quartile and a 1.5-fold increase in the highest weight gain category. Significantly higher odds ratios were noted in postdates patients, hypertensive patients, diabetics, and married patients. Oxytocin induction was less frequent in blacks. After adjusting for these various risk factors, only the higher body mass index groups, the postdates patients, the diabetics, the hypertensives, and the LBW cases had significantly increased risks for induction. Black patients had a decreased risk.

Oxytocin augmentation was used in 16.1% of patients. The odds ratio was significantly increased in the higher weight gain group and the hypertensive patients, with trends noted in association with increasing body mass index, prepregnancy weight, decreasing ma-

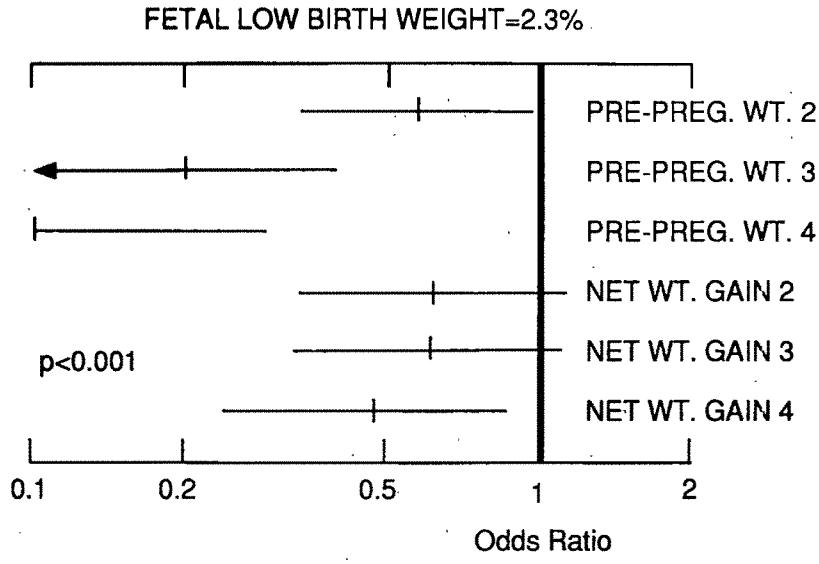


Fig. 6. This graph illustrates prepregnancy weight and net weight gain unadjusted odds ratios and 95% confidence intervals for LBW. There was stepwise reduction in risk of fetal LBW noted with rising prepregnancy weight. Net gestational weight did not demonstrate significant decrease in odds ratio except for those patients gaining >33 pounds. Adjusted trends tests were significant at $p < 0.001$.

ternal height, and the male fetus. The risk was significantly less in the parous patient. After adjusting, only nulliparity was associated with an increased risk of oxytocin augmentation. Teenagers were less likely to receive oxytocin augmentation.

FHR abnormalities. The overall incidence of intrapartum FHR abnormalities was 27% (Table VII). The odds ratio was significantly increased for the highest body mass index category, although maternal prepregnancy weight, maternal height, and maternal gestational weight gain were not associated with increased odds ratios. After adjusting for appropriate covariables, we noted statistically significant odds ratios for body mass index, nulliparity, postdatism, macrosomia, black patients, fetal male gender, and older maternal age. The trends analysis showed no significant association with weight gain and only weak evidence of an association with body mass index ($p = 0.018$). FHR abnormalities were cited as the major cause for cesarean section in about 25% of the unscheduled cesarean sections in this study.

Meconium staining. The overall frequency of meconium staining in the study population was 21.5% (Table VIII). There was a significant increase in the odds ratio noted with the larger body mass index categories, the larger prepregnancy weight quartiles, black patients, unmarried patients, and nulliparous patients. After adjusting for these risk factors, increased risks for meconium staining were found with high prepregnancy weight, short maternal stature, high gestational weight gain, blacks, nulliparous patients, and admitted substance abusers. The trends test indicated an increased risk with weight gain (odds ratio 1.1 for 10 lbs, $p = 0.0003$) and with body mass index (odds ratio 1.22 for 5 units, $p = 0.0001$). Curiously, postdatism and LBW were not associated with significantly increased odds ratios for meconium staining in this study.

Apgar scores and umbilical artery pH. The overall frequency of 1-minute Apgar score ≤7 was 18.3%. Increased odds ratios were noted for increased gestational weight gain, nulliparous patients, and patients with hypertension. These same relationships pertained after adjusting. Black patients were also at greater risk. We found no associations for maternal prepregnancy weight, height, and body mass index. The overall frequency of 5-minute Apgar score ≤7 was 2.5%. There was a significant odds ratio increase in nulliparous patients, teenagers, and patients with hypertension. After adjusting, only nulliparity was associated with an increased risk.

The mean umbilical artery pH −2 SD is 7.12 for this patient population. The overall incidence of a pH <7.12 was 1.5%. No significant associations were noted with body mass index categories, prepregnancy weight, maternal height, or gestational weight gain. A significant association was noted for teenage and nulliparous patients. Adjusting for these findings revealed a significant association only for nulliparous patients.

Resuscitation. Resuscitation, including assisted res-

Table IV. Unscheduled cesarean section

Characteristic	*%*	*Odds ratio and 95% confidence interval*	*p Value*	*Adjusted odds ratio and 95% confidence interval*	*p Value*
Prepregnancy weight quartile (lb)					
1* (≤116)	9.5	—	—	—	—
2 (117-132)	11.2	1.2 (0.87-1.67)	0.25	—	0.87
3 (133-155)	12.8	1.4 (1.02-1.92)	0.034	—	0.11
4 (>155)	14.2	1.6 (1.15-2.14)	0.004	—	0.74
Height (in)					
1* (<62)	15.5	—	—	—	—
2 (62-65)	12.4	0.77 (0.58-1.04)	0.087	0.61 (0.44-0.84)	0.0019
3 (>65)	9.6	0.58 (0.42-0.8)	0.001	0.4 (0.28-0.58)	0.0001
Body mass index category, National Academy of Science					
Low* (<19.8)	8.3	—	—	—	—
Medium (19.8-26)	12.0	1.49 (1.11-2.01)	0.008	—	0.12
High (27-29)	16.4	2.16 (1.46-3.18)	0.0001	1.5 (1.1-2.2)	0.0101
Obese (>29)	14.2	1.82 (1.26-2.6)	0.001	1.38 (1-1.9)	0.043
Weight gain (lb)					
1* (<16)	8.9	—	—	—	—
2 (16-25)	7.8	0.86 (0.56-1.34)	0.51	0.95 (0.6-1.5)	0.82
3 (26-35)	10.3	1.178 (0.8-1.73)	0.400	1.3 (0.86-1.95)	0.21
4 (>35)	16.3	1.988 (1.39-2.83)	0.0001	1.95 (1.32-2.87)	0.0006
Private physician					
No*	11.4	—	—	—	—
Yes	27.5	2.96 (1.92-4.58)	0.0001	2.6 (1.57-4.29)	0.0001
First birth					
Yes*	15.8	—	—	—	—
No	9.1	0.53 (0.43-0.66)	0.0001	0.385 (0.29-0.5)	0.0001
Maternal age (yr)					
<20	10.5	0.895	NS	0.68 (0.5-0.9)	0.009
20-26*	11.6	—	—	—	—
>26	14.3	1.27 (0.98-1.64)	0.07	1.36 (1.0-1.83)	0.04
Birth weight (kg)					
<2.5	14.9	1.64 (0.855-3.16)	0.13	—	0.16
2.5-4.0*	9.6	—	—	—	—
>4.0	27.5	3.57 (2.76-4.6)	0.0001	3.77 (2.8-5.0)	0.0001
Diabetes					
No*	11.6	—	—	—	—
Yes	22.4	2.21 (1.36-3.599)	0.001	1.7 (1-2.9)	0.046
Hypertension					
No*	11.5	—	—	—	—
Yes	19.3	1.84 (1.24-2.74)	0.002	—	0.16
Education					
12th Grade	10.0	0.834 (0.66-1.06)	0.14	—	0.27
12th Grade*	11.7	—	—	—	—
>12th Grade	19.2	1.787 (1.33-2.41)	0.0001	—	0.12

NS, Not significant. Overall incidence 11.9%.
*Reference group.

piration with a mask and oxygen or intubation, was reported with a frequency of 24.5% in this obstetric population. In mothers with a high body mass index and high gestational weight gain, the odds ratios were significantly increased 1.5-fold. Increases were also noted in delivery of the first child and in the case of fetal macrosomia. Neither prepregnancy weight or maternal height were associated with increased risk of resuscitation. Adjusting for these risk factors failed to demonstrate any statistically significant associations between resuscitation and prepregnancy weight, maternal height, or weight gain during pregnancy.

Comment

This is one of the first studies evaluating pregnancy outcome in regard to the body mass index categories recommended by the National Academy of Sciences.[5] This study addresses a number of the research recommendations made in that publication. It identifies certain effects of overall gestational weight gain within specific ethnic groups and among women of different ages. It examines the effects of weight gain on maternal and perinatal outcomes in markedly and moderately obese women, and it tests the ranges of recommended weight gain against outcomes.

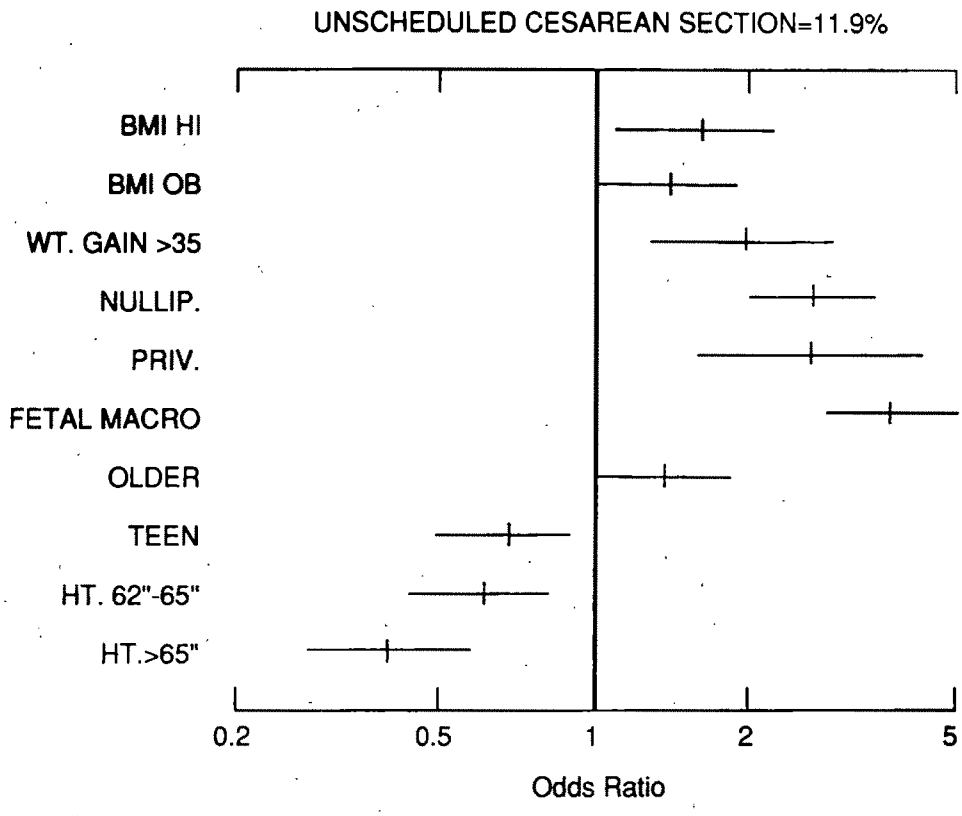


Fig. 7. This graph illustrates odds ratios and 95% confidence intervals for cesarean section, observed for number of demographic, morphologic, and obstetric characteristics after adjustment by stepwise logistic regression. Patients with high body mass index *(BMI)* and high gestational weight gain were noted to have elevated odds ratios. Nulliparous patients, private patients, and older patients, were at increased risk. Patients delivering macrosomic infants appeared to be at greatest risk. Taller patients and teenagers had reduced odds ratios. Adjusted trends tests demonstrated significant risk for unscheduled cesarean section associated with increasing body mass index, maternal weight gain, and birth weight ($p < 0.0001$).

Although this is a longitudinal retrospective study with some of the attendant disadvantages, it would be very difficult to execute a randomized study to assess the effects of maternal weight gain. Relying on patient recall for prepregnancy weight may be considered a limitation of this study, but Susser[14] observed that "patient reported weight has been shown to be reliable within a standard deviation of about 1.5 kg." In comparison with previous studies, the present investigation may be more accurate in that the duration of the pregnancy, and the outcome variables were either determined by measurement or ascertained by attending obstetric staff. Patients with selected medical and surgical illnesses were excluded. Also, this study has the advantage of reflecting recent improvements in obstetric, anesthetic, and pediatric care.

The question of what is the optimal weight gain in pregnancy is clearly a very complex issue. In this study we found that both increasing prepregnancy weight and increasing gestational weight gain were associated with increased frequencies of fetal macrosomia, labor abnormalities, postdatism, meconium staining, and unscheduled cesarean section and with a decreased frequency of LBW. We have elected to focus the discussion mostly on the association of maternal weight gain to birth weight and unscheduled cesarean section rate.

In assessing the clinical implications of the body mass index, it may be important to evaluate maternal weight and height individually because there may be marked variations in these characteristics for women of similar body mass index. For example, in the National Academy of Sciences medium body mass index category, maternal weight could vary from 88 to 202 pounds (229% increase) whereas height could vary from 55.9 inches to 74 inches (132% increase).[5] In the present study, in the adjusted analyses of unscheduled cesarean section, height exhibited a more significant effect than body mass index or prepregnancy weight. Maternal

Table V. Gestational age >42 weeks

Characteristic	%	*Odds ratio and 95% confidence interval*	*p Value*	*Adjusted odds ratio and 95% confidence interval*	*p Value*
Prepregnancy weight quartile (lb)					
1* (≤116)	7.2	—	—	—	—
2 (117-132)	8.5	1.2 (0.84-1.72)	0.31	—	0.68
3 (133-155)	12.7	1.89 (1.34-2.65)	0.0001	1.75 (1.32-2.33)	0.0001
4 (>155)	11.1	1.62 (1.14-2.29)	0.007	1.56 (1.14-2.33)	0.0001
Height (in)					
1 (<62)	6.9	—	—	—	—
2 (62-65)	10.3	1.56 (1.05-2.32)	0.028	—	0.23
3 (>65)	10.4	1.57 (1.04-2.36)	0.0321	—	0.92
Body mass index category, National Academy of Science					
Low* (<19.8)	8.1	—	—	—	—
Medium (19.8-26)	9.7	1.22 (0.896-1.66)	0.207	—	0.06
High (27-29)	12.2	1.58 (1.03-2.4)	0.0341	—	0.13
Obese (>29)	11.5	1.49 (1.01-2.2)	0.043	—	0.025
Weight gain (lb)					
1* (<16)	8.5	—	—	—	—
2 (16-25)	8.3	0.98 (0.63-1.5)	0.913	1.03 (0.65-1.62)	0.89
3 (26-35)	9.1	1.08 (0.73-1.61)	0.693	1.16 (0.76-1.76)	0.47
4 (>35)	11.7	1.42 (0.98-2.06)	0.06	1.33 (0.90-1.98)	0.14
Weight gain, National Academy of Science					
Obese plus high	11.9	1.7 (1.27-2.35)	0.0001	—	—
Within range	9.5	1.3 (0.97-1.9)	0.075	—	—
Low*	7.2	—	—	—	—
Ethnic group					
White*	11.9	—	—	—	—
Black	7.0	0.553 (0.43-0.71)	0.0001	0.64 (0.48-0.85)	0.0016
Other	3.6	0.28 (0.7-1.15)	0.059	—	0.08
Private physician					
No*	10.1	—	—	—	—
Yes	3.7	0.34 (0.12-0.93)	0.028	0.28 (0.1-0.79)	0.014
Married					
No*	8.7	—	—	—	—
Yes	11.2	1.31 (1.04-1.65)	0.023	—	0.54
Tobacco, alcohol, drugs					
No*	8.7	—	—	—	—
Yes	11.9	1.42 (1.122-1.8)	0.003	1.4 (1.08-1.82)	0.0085
Maternal age (yr)					
<20	9.4	0.83 (0.63-1.09)	0.171	—	0.77
20-26*	11.1	—	—	—	—
>26	7.9	0.69 (0.5-0.94)	0.018	0.67 (0.49-0.91)	0.008
Birth weight (kg)					
<2.5	0.0	—	—	—	—
2.5-4.0*	9.1	—	—	—	—
>4.0	17.0	2.04 (1.52-2.75)	0.0001	1.9 (1.37-2.6)	0.0001

Overall incidence 9.8%.
*Reference group.

height probably serves as a better reflection of pelvic size when analyzed alone than when expressed in the ratio that defines body mass index.

A marked increase in fetal macrosomia was noted with increasing maternal body mass index. The adjusted odds ratios for maternal weight gain and prepregnancy weight were both markedly elevated. The model used for adjustments assumed that these characteristics are additive, because no interactions were detected. If these associations were additive, women with a body mass index >29 who gain >30 pounds during pregnancy would be predicted to have a fourfold to fivefold increase in the odds ratio for macrosomia in comparison with the reference population. These preliminary findings suggest that it might be advisable to limit weight gain in obese pregnant women. These results are at variance with previous studies reporting that in obese women weight gain did not influence birth weight. However, several of these studies did not adjust for medical-surgical illnesses and were characterized by smaller numbers.[6, 11] Their results might have been influenced by type II statistical error.

Table VI. Labor abnormalities

Characteristic	*%*	*Odds ratio and 95% confidence interval*	*p Value*	*Adjusted odds ratio and 95% confidence interval*	*p Value*
Prepregnancy weight quartile (lb)					
1* (≤116)	6.4	—	—	—	—
2 (117-132)	7.9	1.26 (0.86-1.85)	0.23	—	0.88
3 (133-155)	7.9	1.26 (0.86-1.85)	0.24	—	0.72
4 (>155)	9.0	1.46 (1.003-2.13)	0.05	—	0.12
Height (in)					
1* (<62)	9.0	—	—	—	—
2 (62-65)	8.6	0.96 (0.66-1.38)	0.81	—	0.20
3 (>65)	6.0	0.65 (0.43-0.98)	0.04	0.57 (0.42-0.78)	0.0004
Body mass index category, National Academy of Science					
Low* (<19.8)	6.2	—	—	—	—
Medium (19.8-26)	7.7	1.26 (0.89-1.79)	0.19	—	0.42
High (27-29)	10.8	1.78 (1.11-2.81)	0.015	—	0.10
Obese (>29)	8.7	1.45 (0.94-2.25)	0.09	—	0.34
Weight gain (lb)					
1* (<16)	6.0	—	—	—	—
2 (16-25)	5.5	0.918 (0.55-1.54)	0.746	0.96 (0.56-1.65)	0.89
3 (26-35)	7.2	1.223 (0.77-1.93)	0.388	1.22 (0.76-1.97)	0.40
4 (>35)	10.0	1.746 (1.14-2.67)	0.010	1.53 (0.97-2.41)	0.059
Ethnic group					
White*	7.2	—	—	—	
Black	8.6	1.22 (0.93-1.59)	0.14	1.68 (1.26-2.25)	0.0003
Other	7.4	1.03 (0.37-2.9)	0.95	—	0.82
Married					
No*	8.6	—	—	—	—
Yes	6.7	0.77 (0.59-1.003)	0.05	—	0.08
Private physician					
No*	7.4	—	—	—	—
Yes	17.0	2.54 (1.5-4.3)	0.0001	2.63 (1.48-4.68)	0.0008
Parity					
1*	11.3	—	—	—	—
>1	5.2	0.44 (0.33-0.57)	0.0001	0.34 (0.25-0.46)	0.0001
Fetal sex male					
No*	6.5	—	—	—	—
Yes	9.0	1.42 (1.09-1.85)	0.009	1.36 (1.03-1.80)	0.02
Maternal age (yr)					
<20	7.7	0.99 (0.67-1.22)	0.50	0.65 (0.47-0.91)	0.01
20-26*	8.5	—	—	—	—
>26	6.3	0.73 (0.51-1.04)	0.08	—	0.81
Hypertension					
No*	7.5	—	—	—	—
Yes	12.9	1.84 (1.15-2.94)	0.009	—	0.16
Birth weight (kg)					
<2.5	1.4	0.188 (0.026-1.36)	0.06	—	0.06
2.5-4.0*	6.8	—	—	—	—
>4.0	16.3	2.679 (1.96-3.67)	0.0001	3.18 (2.25-4.495)	0.0001

Overall incidence 7.8%.
*Reference group.

The increased risks associated with high prepregnancy weight and high weight gain during pregnancy observed in this study have been amply demonstrated in many previous studies.[3, 9, 10, 13] Yet the recent National Academy of Science recommendations advocate increasing maternal weight gain during pregnancy. These recommendations appear to be based primarily on earlier observations that (1) LBW is associated with increased perinatal mortality and infant morbidity and (2) increased maternal weight gain is associated with lower rates of LBW. The inference has been drawn that increasing maternal weight gain will reduce the frequency of LBW and thereby reduce perinatal mortality and morbidity.[5] These assumptions may be dangerous and costly oversimplifications.

The causes of LBW are myriad and some are more hazardous than others.[15, 16] Prematurity accounts for a significant portion of LBW babies. A major portion of LBW term infants have no underlying fetal or maternal abnormalities. These infants are born small for familial

Table VII. FHR abnormalities

Characteristic	*%*	*Odds ratio and 95% confidence interval*	*p Value*	*Adjusted odds ratio and 95% confidence interval*	*p Value*
Prepregnancy weight quartile (lb)					
1* (≤116)	25.8	—	—	—	—
2 (117-132)	26.3	1.025 (0.82-1.28)	0.83	—	0.92
3 (133-155)	26.4	1.033 (0.83-1.29)	0.78	—	0.98
4 (>155)	29.5	1.207 (0.97-1.51)	0.10	—	0.52
Height (in)					
1* (<62)	31.4	—	—	—	
2 (62-65)	26.9	0.806 (0.64-1.01)	0.06	—	0.88
3 (>65)	25.2	0.736 (0.58-0.94)	0.01	—	0.08
Body mass index category, National Academy of Science					
Low* (<19.8)	23.0	—	—	—	—
Medium (19.8-26)	27.2	1.25 (1.023-1.54)	0.03	—	0.11
High (27-29)	29.1	1.38 (1.029-1.85)	0.03	—	0.17
Obese (>29)	31.0	1.506 (1.164-1.95)	0.002	1.33 (1.06-1.67)	0.01
Weight gain (lb)					
1* (<16)	21.6	—	—	—	—
2 (16-25)	19.4	0.96 (0.73-1.26)	0.771	—	0.83
3 (26-35)	18.3	1.09 (0.86-1.38)	0.489	—	0.67
4 (>35)	25.0	1.09 (0.86-1.38)	0.489	—	0.29
Ethnic group					
White*	24.9	—	—	—	—
Black	30.2	1.30 (1.108-1.53)	0.001	1.36 (1.15-1.61)	0.0003
Other	22.2	0.86 (0.45-1.65)	0.65	—	0.89
Gestational age ≥42 wk					
No*	25.9	—	—	—	—
Yes	37.1	1.69 (1.32-2.16)	0.0001	1.84 (1.42-2.38)	0.0001
Private physician					
No*	26.7	—	—	—	—
Yes	34.0	1.41 (0.94-2.13)	0.10	—	0.08
Parity					
1*	32.6	—	—	—	—
>1	22.9	0.616 (0.53-0.72)	0.0001	0.55 (0.46-0.66)	0.0001
Fetal sex male					
No*	24.0	—	—	—	—
Yes	29.9	1.355 (1.16-1.59)	0.0001	1.38 (1.17-1.63)	0.0001
Maternal age (yr)					
<20	29.3	1.25 (1.04-1.50)	0.02	—	0.93
20-26*	24.8	—	—	—	—
>26	28.4	1.198 (0.98-1.46)	0.08	1.385 (1.129-1.699)	0.0014
Hypertension					
No*	26.5	—	—	—	—
Yes	35.9	1.55 (1.13-2.15)	0.007	—	0.12
Birth weight (kg)					
<2.5	31.1	1.179 (0.715-1.94)	0.52	—	0.28
2.5-4.0*	27.7	—	—	—	—
>4.0	20.9	0.69 (0.531-0.901)	0.006	0.65 (0.49-0.87)	0.003

Overall incidence 27%.
*Reference group.

or constitutional reasons. In the absence of asphyxial insults, which are mostly preventable, these babies have excellent outcomes.[15, 17] An increase in maternal weight gain may reduce the frequency of this type of LBW infant, but there is little if any evidence of an associated improvement in perinatal mortality or morbidity.

Intrinsic abnormalities in the fetus (congenital anomalies, infections, chromosomal abnormalities, etc.) account for a small percentage of LBW infants but contribute significantly to poor outcomes, including death and morbidity. Maternal illnesses account for another portion of LBW infants, and asphyxial complications are primarily responsible for the poorer outcome noted in these cases. Unfortunately, in prior studies of the mortality and morbidity associated with LBW, the consequences of prematurity, intrinsic fetal abnormalities, and asphyxial events were not taken into consideration. There is little experimental or clinical evidence that increased maternal weight gain in pregnancy will reduce the wastage associated with these forms of LBW.

Table VIII. Meconium-stained amniotic fluid

Characteristic	*%*	*Odds ratio and 95% confidence interval*	*p Value*	*Adjusted odds ratio and 95% confidence interval*	*p Value*
Prepregnancy weight quartile (lb)					
1* (≤116)	17.7	—	—	—	—
2 (117-132)	21.0	1.237 (0.97-1.59)	0.09	1.29 (0.99-1.68)	0.49
3 (133-155)	22.5	1.348 (1.05-1.73)	0.02	1.44 (1.11-1.87)	0.005
4 (>155)	25.2	1.571 (1.23-2.00)	0.0001	1.71 (1.3-2.2)	0.0001
Height (in)					
1* (<62)	21.7	—	—	—	—
2 (62-65)	23.1	1.08 (0.84-1.39)	0.54	—	0.61
3 (>65)	19.1	0.849 (0.65-1.11)	0.24	0.70 (0.58-0.85)	0.0003
Body mass index category, National Academy of Science					
Low* (<19.8)	16.5	—	—	—	—
Medium (19.8-26)	21.6	1.39 (1.11-1.75)	0.004	—	0.94
High (27-29)	26.7	1.84 (1.35-2.52)	0.0001	—	0.37
Obese (>29)	25.8	1.756 (1.33-2.33)	0.0001	—	0.89
Weight gain (lb)					
1* (<16)	21.6	—	—	—	—
2 (16-25)	19.4	0.874	0.38	0.99 (0.73-1.36)	0.98
3 (26-35)	18.3	0.816	0.15	0.99 (0.74-1.34)	0.98
4 (>35)	25.0	1.214	0.14	1.44 (1.09-1.89)	0.009
Ethnic group					
White*	18.8	—	—	—	—
Black	25.7	1.5 (1.3-1.49)	0.0001	1.70 (1.41-2.06)	0.0001
Other	18.5	0.98	0.95	—	0.65
Married					
No*	23.8	—	—	—	—
Yes	18.8	0.737 (0.62-0.88)	0.001	—	0.14
Tobacco, alcohol, drugs					
No*	21.1	—	—	—	—
Yes	22.4	1.081	0.39	1.29 (1.07-1.58)	0.008
Parity					
1*	23.8	—	—	—	—
>1	19.9	0.797 (0.67-0.95)	0.009	0.7 (0.58-0.85)	0.0002
Maternal age (yr)					
<20	22.7	1.19 (0.98-1.46)	0.08	—	0.92
20-26*	19.8	—	—	—	—
>26	23.7	1.26 (1.02-1.56)	0.03	1.33 (1.06-1.66)	0.012
Education					
<12th Grade	22.9	1.143	0.14	1.21 (1.00-1.45)	0.04
12th Grade*	20.5	—	—	—	—
>12th Grade	20.4	0.99	0.94	—	0.76
Birth weight (kg)					
<2.5	16.2	0.72 (0.39-1.34)	0.30	—	0.28
2.5-4.0*	21.2	—	—	—	—
>4.0	25.3	1.253 (0.98-1.62)	0.07	1.31 (0.99-1.7)	0.046

Overall incidence 21.5%.
*Reference group.

In fact, Kleinman[18] in a study of 3946 cases of white non-Hispanic women found that total maternal weight gain tended to overstate the association between maternal weight gain and fetal growth. He recommended the use of net weight gain rate (total maternal weight gain minus baby's birth weight divided by weeks of gestation) to assess these interrelationships. With this measure of maternal weight gain he found that an increased net weight gain was linked to a significant decrease in the frequency of term LBW infants only in women of moderate body mass index (19.8 to 26.0). In obese patients (body mass index >26.0), an increased net weight gain tended to increase the frequency of term LBW infants. Increases in net weight gain appeared to have little impact on the frequency of preterm LBW, regardless of the mother's body mass index.

In the present study we attempted to estimate the public health implications of our findings by predicting the national costs, in terms of rates of unscheduled cesarean section and low birth weight relative to differences in maternal weight gain. The recent National Academy of Science recommendations for weight gain in pregnancy[5] specify 28 to 40 pounds for a body mass index <19.8, 25 to 35 pounds for a body mass index

of 19.8 to 26, 15 to 25 pounds for a body mass index of 27 to 29, and at least 15 pounds for a body mass index >26 to 29. With these ranges (and 15 to 25 pound range for a body mass index >29) we calculated the cesarean section rates for patients according to whether their weight gain was below, within range, or above the National Academy of Science recommendations. The unscheduled cesarean section rates were 7.7%, 9.6%, and 16.6%, respectively, for these three groups. With multiple logistic regression we found that if weight gain was limited in all cases to the maximum National Academy of Science recommendation, the predicted unscheduled section rate was 9.7% (Table IX). If all weight gain was limited to the lowest NAS recommendation, the predicted unscheduled section rate was 8.1%. Assuming our statistical model is correct and that our patient population reflects the national situation, a decrease in cesarean section rate from 11.9% to 8.1% nationally could result in annual savings of $253 to $436 million dollars.[19]

A theoretic reduction in body mass index values to a maximum of 29 or even 26 would be expected to reduce the unscheduled cesarean section rate by only 0.4% to 0.9% (Table IX). The greater effect of limiting gestational weight gain is due to the higher prevalence of excessive gestational weight gain compared with high body mass index in this population.

These theoretic reductions in maternal weight would be predicted to also increase the frequencies of LBW. The LBW rates associated with maternal weight gain below, within range, or above National Academy of Science recommendations were 4.7%, 1.8%, and 1.1%, respectively. Again with multiple logistic regression, we found that limiting all weight gain to the maximum National Academy of Science recommendations yielded a predicted LBW rate of 2.5% (Table X). If all weight gain was limited to the lowest National Academy of Science recommendation, the predicted LBW frequency was 3.0%. Again, the predicted influence of reduced body mass index is a lesser increase in LBW frequency. Limiting body mass index to a maximum of 29 or 26 results in a predicted increase in LBW rate of only 0.1% to 0.2% (Table X).

Assuming our statistical model is correct and that this study population reflects the national experience with LBW, we estimate that an increase in the national frequency of this type of low birth weight from 2.3% to 3.3% would result in additional hospital expenses of about $1 to $2 million dollars per annum. Recent studies have shown no increase in long-term morbidity.[17, 20, 21] The net effect appears to argue strongly for limiting weight gain in pregnancy.

In summary, the results of this study indicate that either high prepregnancy weight or high weight gain during pregnancy lead to increased cesarean sections and other complications. These complications seem to outweigh any purported medical benefits. Furthermore, the additional expenses required to deal with these consequences on a national basis could be substantial. Recent recommendations advising additional weight gain in pregnancy need further evaluation before implementation.

Table IX. Predicted unscheduled cesarean section rates with decreased weight gain and body mass index

	Body mass index		
Weight gain	*Observed (%)*	*Maximum of 29 (%)*	*Maximum of 26 (%)*
Observed	11.9	11.4	11.0
Compliant	9.7	9.3	9.0
Low	8.1	7.7	7.5

Table X. Predicted LBW rates according to gestational weight gain and body mass index

	Body mass index		
Weight gain	*Observed (%)*	*Maximum of 29 (%)*	*Maximum of 26 (%)*
Observed	2.3	2.4	2.5
Compliant	2.5	2.6	2.7
Low	3.0	3.1	3.3

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

Dr. Barry Gross, Newport News, Virginia. Johnson et al. examine carefully the emphasis placed in recent years on the notion that "more is better" when referring to weight gain in pregnancy. It has been widely held that this emphasis on maximizing pregnancy weight gain reached its pinnacle in 1989 with the publication of results from the National Natality Study by the National Center for Health Statistics.[1] It was noted in that publication that LBW was 2.3 times more likely, and fetal death 1.5 times more likely, in women who gained <20 pounds during pregnancy when compared with those who gained >20 pounds. In addition, it was noted that 20% of all black mothers in 1980 gained <16 pounds during pregnancy and had the poorest pregnancy outcomes. These results might lead one to believe that there is a direct correlation between the amount of weight gained in pregnancy and the health of the baby delivered and that increasing the amount of weight gained in pregnancy will reduce the incidence of LBW infants. Johnson et al. have demonstrated that while this may indeed be true in women who start pregnancy at lower body weights, it does not translate with the same validity to those women who begin pregnancy overweight.

The first published study of diet and pregnancy was by Prochownick[2] in 1901. This work demonstrated that restricted food intake throughout pregnancy reduced the birth weights of males by approximately 400 gm and those of females by 500 gm. Indeed, over the past century there have been many varying changes in recommendations regarding weight gain in pregnancy. Over most of this time the recurrent theme was restriction of caloric intake, because it was felt that this restriction could prevent large babies and associated difficult labors and deliveries.[3, 4] This approach is entirely understandable because maternal mortality was very high and delivery by cesarean section was often an act of desperation. Later in the century controlling weight gain during pregnancy was advocated to prevent toxemia.[5]

Studies published in the 1950s, 1960s, and early 1970s generally indicated that an average weight gain of 12.5 kg (27.5 lb) was considered normal. In 1970 the Food and Nutrition Board's Committee on Maternal Nutrition and, a few years later, the American College of Obstetricians and Gynecologists published the average desirable weight gain as 24 pounds.[6, 7] In fact, the recommended gestational weight gains have essentially doubled between 1930 and 1990, from 15 pounds in 1930 to 30 pounds in the late 1980s.

Before publication of the National Natality Study,[8] generally recommended weight gain was on the order of 10 to 12 kg (22 to 27 pounds) during pregnancy. Mirroring the findings of that study, most publications in obstetrics at that time, including Standards of the American College of Obstetricians and Gynecologists, generally noted that regardless of how much women weigh before they become pregnant, the gaining of between 26 and 30 pounds during pregnancy could improve the outcome of pregnancy and reduce their chances for having the pregnancy end with fetal morbidity.[9]

There is little doubt that the plight of the LBW infant has deserved the highest order of attention by our specialty. A large body of evidence indicates that the amount of gestational weight gain is a determinant of fetal growth.[10] Lower total weight gains are associated with an increased risk of LBW babies with higher perinatal mortality. In this paper, Johnson et al., have refocused our attention to the opposite end of the continuum, excessive maternal weight gain and its outcome. Both increasing maternal prepregnancy weight and increasing maternal gestational weight gain were found to be associated with increased frequencies of fetal macrosomia, labor abnormalities, FHR abnormalities, postdatism, meconium staining, and unscheduled cesarean section.

Indeed, most studies have demonstrated an increased risk of hypertension, preeclampsia, and gestational diabetes in patients manifesting excessive maternal weight gain. Babies are bigger, and cesarean section rates are higher. Higher birth weights are associated with prolonged labor, shoulder dystocia, birth trauma, and asphyxia. Many believe that postpartum hemorrhage, puerperal infection, and venous thrombosis are more common. Surgical morbidity and mortality rises, because patients are more difficult to administer anesthesia to, have more postanesthesia complications, and have more wound complications.

Although the encouraging of weight gain has been seen as a means by which we might lower the number of LBW babies, it is clear that the weight that is gained is not always either nutritionally adequate or appropriate. Weight gain is often not a reflection of a nutritionally balanced diet.[10]

Johnson et al. used both actual maternal weight gain and body mass index as tools in this evaluation. Of interest is body mass index, which is defined as prepregnancy weight in kilograms divided by height in meters squared (or "weight for height") and has been endorsed by the National Academy of Science, in their 1990 publication *Nutrition During Pregnancy*, as a tool for patient evaluation. Johnson et al.'s data appear to demonstrate what others have noted, e.g., that in the initial risk assessment of outcomes related to maternal weight, the calculation of maternal body mass index offers no advantage over simply weighing the patient.[11]

It is well known that the effect of gestational weight gain on fetal growth is modified by the mother's prepregnancy weight for height. Multiple studies have demonstrated that the effect of a given weight gain (or rate of weight gain) on fetal growth is greatest in thin women and least in overweight women.[12, 13] Prepregnancy weight for height, or body mass index, can be a determinant of fetal growth independent of the effect of gestational weight gain. Studies show that women who are thinner before pregnancy tend to have babies that are smaller than those of their heavier counterparts with the same gestational weight gain. A desirable pregnancy weight gain seems to be, in general, inversely proportional to the body mass index of the patient.[12] Studies also suggest that increases in maternal fat, lean tissue, and body water may each be associated with increased fetal growth. Problems associated with this increased fetal growth, as demonstrated by Johnson et al., appear to be more pronounced in short women (high body mass index).

The data presented by the authors clearly suggests that there are many good reasons to suggest limitations on weight gain in selected pregnancies. Although this principle is at variance with many of the nutritional guidelines published in the mid 1980s, it does agree with the most recent (1990) suggestions by the National Academy of Science. The Academy has divided all pregnancies into four groups by determining prepregnancy weights-for-heights. As one might expect, as the individual groups' body mass index increased, its suggested weight gain decreased. Individualization of weight gain and appropriate dietary instruction therefore would seem to be the prescribed manner to approach the topic of appropriate pregnancy weight gain.

Although the degree of weight gain restriction in the overweight pregnant woman may be debated (as it has over the past 91 years), it is clear from this study by Johnson et al. that only by aggressively managing the weight gain of patients from both ends of the prepregnancy weight spectrum and pregnancy weight gain spectrum will we be able to reduce significantly maternal and neonatal morbidity and mortality and the associated physical and financial resources that those morbidities exhaust.

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Dr. Herbert G. Hopwood, Jr., Arlington, Virginia. I would like to ask Dr. Johnson if he had an increase in shoulder dystocia in his patients compared with his controls?

Dr. Gene Burkett, Miami, Florida. The bottom line is what is the ideal pregnancy weight gain? Have you looked at the prepregnancy weight differences with the postpartum weight and related that to outcome as a means of trying to devise in your four groups what the ideal prepregnancy weight gain for each group might be?

Dr. John F. Huddleston, Atlanta, Georgia. Many of the studies associated with weight gain in pregnancy seem to take the extremes in weight gain rather than

looking at the time course of weight gain. I think from a clinical point of view many of us are more concerned with the abrupt weight gain than the steady weight gain. I wonder if the time course was considered in this study.

DR. JOHN J. BRITTON, Sumpter, South Carolina. Obviously, with increasing postdatism we get more fetal macrosomia and meconium staining of the amniotic fluid, but the data seem to indicate that excessive maternal weight gain during pregnancy or the obese patient who was obese before pregnancy tends to increase postdatism. Why does this group appear to have an increase in postdatism?

DR. JOHNSON (Closing). The question of what is the optimal weight gain in pregnancy is an emotional and controversial issue; and it has been for a very long time, probably because of its complexity.

We found the incidence of shoulder dystocia to be significantly increased if there was macrosomia. That seemed to be the major determinant as to whether shoulder dystocia was diagnosed. We do not have analyses that would lead us to conclude what is the optimal prepregnancy weight. I think that the National Academy of Science has chosen ideal body weight, the basis of the Metropolitan Life Insurance data of 1959, to be a body mass index of 19 to 26, that is, 90% to 110% of ideal body weight.

Dr. Huddleston raised an interesting point about the time course of weight gain during pregnancy. We did not look at that in our population. That would be a difficult and time-consuming task because we cannot retrieve that information from our computerized data bank. Dr. Kleinman looked at the rate of weight gain during pregnancy and did not report any birth weight differences from those that we have reported here.[1]

Why an increase in postdatism occurs is a very good question. We have tried to analyze this from our data. It does occur if the mother enters pregnancy above ideal weight or if she gains excessive weight during the course of the pregnancy. The increase in postdatism persists even after adjusting for macrosomia. We do not have a ready explanation other than it appears that excessive weight leads to prolonged pregnancy, rather than the converse.

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A prospective randomized clinical trial comparing two oxytocin induction protocols

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OBJECTIVE: Our objective was to compare the safety and efficacy of two accepted oxytocin induction protocols that differ in oxytocin dose increments.
STUDY DESIGN: At the Carolinas Medical Center in Charlotte, North Carolina, 151 women with indications for induction of labor were prospectively randomized into one of two oxytocin induction protocols. Safety and efficacy of the two protocols were analyzed with two-tailed *t* tests and χ^2.
RESULTS: Time from induction to establishment of a regular labor pattern was significantly shorter in the experimental group compared with the traditional group ($p = 0.03$). However, no significant difference was seen from onset of induction to time of delivery. Incidences of hyperstimulation were not significantly different between the two protocols, but there was a trend toward a higher incidence of fetal heart rate changes in the experimental group ($p = 0.08$).
CONCLUSION: These data suggest that induction with larger dose increments will shorten time to adequate labor without an associated increase in uterine hyperstimulation or poor neonatal outcome. The differences in heart rate changes are concerning and merit further investigation. If confirmed by further studies, an increased risk of cord compression could outweigh the benefit of a faster onset of contractions.
(Am J Obstet Gynecol 1992;167:373-81.)

Key words: Oxytocin, labor

Since Theobald et al.[1] first described the use of continuous intravenous oxytocin in 1948, multiple protocols have been advocated by various authors for the use of oxytocin in the induction of labor. These oxytocin induction protocols vary in the initial dose, the time interval between dose increases, and the size of the dose increase.

Seitchik et al.[2] demonstrated in vivo that oxytocin concentrations reach steady state after 30 to 40 minutes. This finding has led to the formation of oxytocin induction protocols with interval increases of ≥30 minutes,[3-5] including the protocol listed by The American College of Obstetricians and Gynecologists (ACOG) Technical Bulletin No. 110.[6] The University of Texas Health Science Center in San Antonio presented a similar time interval between increases of continuous oxytocin, but it varied from the ACOG protocol by its larger rate increments.[7] The question arises as to whether these larger rate increments, which obtain higher oxytocin dosages sooner, may shorten the time from induction to labor and delivery without increasing the risk to the fetus.

The purpose of our study was to compare two oxytocin induction protocols: the one listed by ACOG and one similar to that presented by the University of Texas Health Science Center in San Antonio. Safety and efficacy were assessed for each protocol by comparing hyperstimulation, fetal distress, time from induction to labor, and time from induction to delivery.

Material and methods

This randomized, prospective study was performed at the Carolinas Medical Center after the approval of the Institutional Review Board of the medical center. A total of 151 patients with indication for induction of labor consented to participate in the study and were randomized to one of the two induction protocols described below. Randomization was performed only after the patient agreed to be in this clinical trial and was by computer-generated random assignment with sealed, opaque envelopes. Clinical management was by house officers with immediate inhouse consultation available at all times.

Inclusion criteria were a gestational age of 37 to 42 weeks, cephalic presentation, an adequate pelvis by clinical pelvimetry, no more than one prior lower segment cesarean section, and premature rupture of membranes with patient not in labor. Patients not in labor who received prostaglandin gels for cervical ripening

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were randomized after receiving the gels. Labor was defined as regular contractions with evidence of cervical change (dilatation or effacement).[8] Excluded from the study were women with an abnormal presentation, abruptio placentae, placenta previa, previous vertical cesarean section, unknown uterine scar, previous myotomy, twins, polyhydramnios, active genital herpes, magnesium sulfate therapy, estimated fetal weight ≥4500 gm, nonreassuring fetal testing results, and regular uterine contractions (patients already contracting were considered augmentation of labor).

A total of 76 patients were randomized to the traditional protocol, which was modeled after ACOG Technical Bulletin No. 110[6] and which is currently used at our center. This will be referred to as the traditional protocol. Oxytocin was started at 1 to 2 mU/min and was gradually increased in dose increments of 1 to 2 mU/min at 30-minute intervals as needed. The rate was increased as needed according to the judgment of the nurses and physicians at the bedside. A total of 75 patients were randomized to the experimental protocol, a protocol similar to that described by Blanco and Finley.[7] This will be referred to as the experimental protocol. Oxytocin was started at 1 to 2 mU/min, and each new oxytocin rate was doubled from the preexisting rate (for example, 1, 2, 4, 8, 16 mU/min). An interval of 40 minutes between increases was used. After a level of 8 mU/min was reached, less than doubling the rate was acceptable if progression to hyperstimulation was feared (for example, 8, 10, 12 mU/min).

For both protocols 20 IU of synthetic oxytocin (Lyphomed, Fujisawa USA, Deerfield, Ill.) was mixed in 1 L 5% dextrose in lactated Ringers' solution and delivered by a secondary intravenous line. Oxytocin infusion rate was controlled by continuous electronic infusion pump. Continuous electronic fetal heart rate (FHR) and contraction monitoring were performed in all patients. Amniotomy was performed and an intrauterine pressure catheter was placed by the supervising physician when clinically indicated. Oxytocin was decreased for hyperstimulation, defined as five or more contractions in 10 minutes, contraction duration >90 seconds, or baseline uterine tone of 25 mm Hg. Oxytocin was temporarily discontinued for nonreassuring fetal heart rate changes, which included severe variable decelerations, recurrent late decelerations, prolonged decelerations, or loss of beat-to-beat variability. After 8 to 10 hours of oxytocin, if the membranes were intact and the cervix was still unfavorable, oxytocin was discontinued and the patient was scheduled for a second or third day of induction to be performed the following day. A Bishop score was documented at the beginning of each induction day.[9]

Epidural anesthesia was not withheld from patients participating in this study. The same group of house officers used the same continuous-infusion, lumbar epidural technique for patients in both protocols. Assisted vaginal deliveries were done only for clinical indication.

Patient demographics, such as age, race, gravidity, parity, and gestational age and clinical criteria (indication for induction, Bishop score, membrane status, cervical dilatation, prostaglandin gels application) were obtained from each patient on admission to the study. Obstetric outcome (number of days for induction, method of delivery, complications associated with delivery) and neonatal outcome (Apgar scores and cord pH) were recorded at completion of induction. Type and frequency of hyperstimulation episodes and FHR patterns were noted by reviewing each fetal monitor strip. Temporal data on each patient included onset of induction to time of delivery and to time of labor. Labor was defined as >150 Montevideo units as measured by an intrauterine pressure catheter. When technical consideration prevented the use of an intrauterine pressure catheter, labor was defined as three palpably strong contractions each lasting 60 seconds during a 10-minute interval. Timing of rupture of membranes and placement of epidural anesthesia were recorded. For analysis the timing of epidural placement was categorized according to placement before or after a regular labor pattern was established. If patients required more than 1 day of induction, then time to labor and time to delivery were calculated with data from the last day of induction only.

Sample size determination was based on the detection of a 2-hour difference in total length of labor between the two groups. Assuming a standard deviation of 4 hours in total length of labor, an 80% power, and an alpha level of 0.05, a total of 63 women in each group was needed.[10] A 10% failure rate was assumed, which necessitated increasing the enrollment to a minimum of 70 women in each group for a total sample size of 140.

Two-tailed t tests were used to determine significant differences between the two treatment groups for all continuous variables. Significant associations between categoric variables was determined with χ^2 analysis. Once significant univariate associations were determined, then stepwise multiple regression was used to examine temporal outcomes multivariately. Stepwise logistic regression was used to examine the outcomes of hyperstimulation and abnormal FHR patterns. Throughout all analysis a p value of 0.05 was used for the level of significance.

Results

A total of 151 patients were randomized according to the protocol, but there were 13 patients who had to be deleted from the study because of patient withdrawal, placement on regimen of intravenous magne-

Table I. Patient demographics comparing oxytocin induction groups

	Traditional protocol (n = 68)	*Experimental protocol (n = 70)*	*p Value*
Age (yr, mean ± SD)	22.3 ± 5.3	22.7 ± 5.7	0.72
Gestational age (wk, mean ± SD)	40.6 ± 1.5	40.5 ± 1.6	0.89
Race (No.)			
Nonwhite	43 (63.2%)	37 (52.9%)	0.24
White	25 (36.8%)	33 (47.1%)	
Parity (No.)			
Primigravid	45 (66.2%)	46 (65.7%)	0.95
Multiparous	23 (33.8%)	24 (34.3%)	
Previous cesarean section (No.)			
Yes	4 (5.9%)	4 (5.7%)	0.56
No	64 (94.1%)	66 (94.3%)	

Table II. Indications for oxytocin induction

	Traditional protocol (n = 68)		*Experimental protocol (n = 70)*	
	No.	*%*	*No.*	*%*
Postdate pregnancy	25	36.8	30	42.9
Premature rupture of membranes	23	33.8	20	28.6
Maternal medical indications	4	5.9	7	10.0
Intrauterine growth retardation	1	1.5	1	1.4
Impending macrosomia	2	2.9	0	0
Gestational diabetes	13	19.1	9	12.9
Oligohydramnios	0	0	3	4.3

sium sulfate for worsening preeclampsia, or failure to meet the inclusion criteria. Of the deleted individuals eight had been assigned to the traditional protocol and five to the experimental protocol. A total of 138 patients met the inclusion criteria for the study, with 68 assigned to the traditional protocol and 70 assigned to the experimental protocol.

Patient demographics shown in Table I indicate that there were no significant differences between the two study groups with respect to age, estimated gestational age, race, parity, or vaginal birth after cesarean delivery. The indications for induction of labor are listed in Table II. The most frequent indications in both groups were postdates and premature rupture of membranes, whereas gestational diabetes was more frequent in the traditional protocol group compared with the experimental group.

The clinical characteristics presented in Table III demonstrate no significant differences between the two groups with relation to the Bishop score at the time of enrollment in the study, the Bishop score at the time of induction on the day of delivery, the cervical dilatation at onset of induction, or the status of the membranes on entry to the study. The use of epidural anesthesia, prostaglandin gels for cervical ripening, and intrauterine pressure catheters was similar between the two groups.

The obstetric outcomes outlined in Table IV show that the groups were similar in terms of mean infant birth weight, cord pH, and Apgar scores. There were no 5-minute Apgar scores <8 in either of the two groups. Seventy-five percent of the patients in each group delivered on the first day of induction, with the remainder delivered on the second and third days of induction. The number of second- and third-day inductions were similar between the two protocol groups (Table IV). Examination of the 1-day inductions as compared with the 2- and 3-day inductions indicated that the 1-day inductions achieved adequate labor and delivery sooner, and required significantly less oxytocin. There was no difference, however, in FHR changes or cases of hyperstimulation (not shown).

There was a significant difference between the two study groups in the method of delivery (Table IV). Although the cesarean section rates were similar with the two protocols, there was an increased incidence of assisted vaginal deliveries in the experimental group. In reviewing the labor data on the 24 patients who had assisted vaginal deliveries, 11 patients (one in the traditional group and 10 in the experimental group) underwent assisted deliveries for second stage FHR changes (i.e., severe variables or persistent bradycardia). There were 11 patients (four in the traditional group and seven in the experimental group) who underwent assisted deliveries for maternal exhaustion and two patients in the traditional group who had assisted deliveries for persistent occipitoposterior presentation. Neonatal outcome was not significantly different between the two protocols in the assisted and spontaneous vaginal deliveries with respect to cord pH, birth weight or 5-minute Apgar scores (not shown).

The most frequent indication for cesarean section in both groups was secondary arrest of dilatation or arrest of descent, although fetal distress occurred in five patients and face presentations in five patients (Table IV). Complications were similar between the protocols, and there were a total of five patients who had failed inductions in the study. Three of these patients delivered vaginally 7 to 10 days later (two in the traditional protocol and one in the experimental protocol), and the remaining two required cesarean sections at the time of the failed induction (one in each protocol).

Mild shoulder dystocia occurred in four patients, with three of the four occurring in the experimental group. There were no adverse neonatal outcomes from these four deliveries. Meconium staining and nuchal

Table III. Clinical characteristics of oxytocin induction groups

	Traditional protocol (n = 68)	*Experimental protocol (n = 70)*	*p Value*
Status of membranes at onset of induction (No.)			
Intact	42 (66.8%)	44 (62.9%)	0.90
Ruptured	26 (38.2%)	26 (37.1%)	
Cervical dilatation at onset of induction (cm, mean ± SD)	1.8 ± 1.1	1.7 ± 1.1	0.64
Prostaglandin gels used before induction (No.)			
Yes	38 (55.9%)	41 (58.6%)	
No	30 (44.1%)	29 (41.4%)	0.75
Bishop score at onset of induction (mean ± SD)	5.4 ± 2.3	5.1 ± 2.2	0.40
Bishop score on day of delivery (mean ± SD)	5.5 ± 2.3	5.3 ± 2.1	0.61
Intrauterine pressure catheter (No.)			
Yes	54 (79.4%)	59 (84.3%)	0.46
No	14 (20.6%)	11 (15.7%)	
Epidural anesthesia (No.)			
Yes	43 (63.2%)	40 (57.1%)	0.47
No	25 (36.8%)	30 (42.9%)	

cord were the most frequent complicating events; these occurred equally in the two groups.

According to the stringent definition of uterine hyperstimulation used in this study, there were 38 (60.3%) women in the traditional protocol and 43 (62.9%) in the experimental protocol who were classified as having hyperstimulation (Table V). The types of hyperstimulation are shown in Table VI, with five or more contractions occurring in 10 minutes being the most frequent hyperstimulation pattern. A description of FHR patterns is included in Table VI, with the most commonly occurring FHR change being variable deceleration. Normal FHR patterns throughout labor induction were seen in 41.2% of the women in the traditional protocol group and in 27.1% of the experimental group, with a trend toward significance ($p = 0.08$) (not shown). Although the incidence of uterine hyperstimulation was greater than expected, it was associated with changes in FHR patterns in only 10 of the 40 patients in the traditional group and 15 of the 43 patients in the experimental group (Table V).

Table VII shows that the mean maximum dose of oxytocin in the two groups differed but was not statistically significant ($p = 0.07$). The oxytocin dose at the time labor was attained was similar in the two protocols.

The number of patients who had the oxytocin dosage modified by either decreasing or temporarily discontinuing the infusion due to perceived hyperstimulation or FHR changes did not significantly differ according to oxytocin protocol. If the oxytocin was discontinued temporarily, the average length of time it was off was 1.8 hours in the traditional protocol group and 1.7 hours in the experimental group (not shown). If the oxytocin was temporarily turned off, the mean number of times this occurred during the induction was 1.9 times in each group.

The temporal intervals of labor are presented in Table VIII. These data are only from patients who were delivered vaginally. Excluded were patients who were delivered by cesarean section (13 in the traditional protocol group and 16 in the experimental protocol group) and those three patients with failed inductions who were delivered vaginally at a later date. This part of the analysis used a total of 106 patients with 53 women in each group.

The time from induction to labor was significantly different between the two groups ($p = 0.03$). However, the time from onset of induction to delivery time was not significantly different ($p = 0.33$). There was not a significant difference between the two groups in comparing the timing of epidural anesthesia or rupture of membranes with time of delivery.

On examination of potential confounders it was determined that the time required to establish a regular labor pattern was significantly decreased for women with ruptured membranes but was not significantly affected by epidural anesthesia, the timing of epidural placement, parity, use of prostaglandin gels, or Bishop score. The total length of labor from induction to delivery was significantly decreased for women who were multiparous, had ruptured membranes, did not have epidural anesthesia, or did not have prostaglandin gels placed (not shown). Bishop scores at time of induction and use of epidural anesthesia did not affect the total length of labor.

In the multivariate analysis higher oxytocin dosages at the time of labor, ruptured membranes, timing of the epidural placement, and being assigned to the experimental protocol were all associated with a significantly reduced time for establishment of a regular labor pattern. The total oxytocin dose for the study, use of epidural anesthesia, use of prostaglandin gels, presence of hyperstimulation, and parity were not significantly associated with the time required to achieve a regular

Table IV. Obstetric outcome of oxytocin induction groups

	Traditional protocol (n = 68)	*Experimental protocol (n = 70)*	*p Value*
Birth weight (gm, mean ± SD)	3386 ± 566	3391 ± 531	0.96
Cord pH (mean ± SD)	7.27 ± 0.07	7.26 ± 0.07	0.39
Arterial (No.)	56	57	
Venous (No.)	6	5	
Source unknown (No.)	2	1	
Not obtained (No.)	4	7	
5 min Apgar score	8.9 ± 0.37	8.8 ± 0.41	0.42
Days of induction (No.)			
1	51 (75.0%)	53 (75.7%)	0.58
2	6 (8.8%)	9 (12.9%)	
3	11 (16.2%)	8 (11.4%)	
Delivery method (No.)			
Vaginal	48 (70.6%)	37 (52.9%)	0.05
Assisted vaginal	7 (10.3%)	17 (24.3%)	
Cesarean section	13 (19.1%)	16 (22.8%)	
Indication for cesarean section (No.)			
Dystocia	6 (46.1%)	11 (73.3%)	0.36
Fetal distress	2 (15.4%)	3 (13.3%)	
Failed induction	1 (7.7%)	1 (6.7%)	
Face presentation	4 (30.8%)	1 (6.7%)	
Complications of delivery* (No.)			
Shoulder dystocia	1	3	
Meconium staining	6	8	
Nuchal cord	9	10	
Other†	11	9	
No complications	42	40	

*More than one complication may be reported for some patients.

†Includes pregnancy-induced hypertension (n = 6), postpartum hemorrhage (n = 1), malpresentation (n = 4), suspected chorioamnionitits (n = 6), uterine inversion (n = 1), precipitate delivery (n = 1), anxiety (n = 1).

labor pattern. The only variables that contributed significantly toward the reduction in total length of labor were higher total oxytocin doses for the study, ruptured membranes, and parity. Epidural placement and use of prostaglandin gel did not significantly affect the total length of labor in the adjusted analysis.

Multiple logistic regression was used to adjust for potential confounders while assessing the relationship between oxytocin protocols and the outcomes of hyperstimulation and FHR patterns. In a stepwise procedure the likelihood ratio test indicated that none of the independent variables contributed significantly to the incidence of hyperstimulation. The placement of an epidural anesthetic was the only variable that contributed significantly to FHR changes.

Comment

The yearly oxytocin induction rate of 5.3% (239/4552) in this center suggests that each year approximately 200,000 American women undergo oxytocin induction of labor. An additional large number of patients undergoes oxytocin augmentation of labor each year. Considering the large number of patients involved, surprisingly few papers have reported clinical trials comparing the several options in technique. Part of the explanation may be that several of the options have proved acceptably safe and effective during extensive clinical usage in particular centers. Nonetheless, in our view there is no reason why these protocols should necessarily be equivalent in any way, and a multiplicity of techniques suggests a need for comparisons to determine which is best. Considering the relative paucity of data comparing several widely used protocols, in our opinion the recent revision of ACOG Technical Bulletin No.157[11] (1991, revised from no.110,1987) appropriately expanded its list of proffered techniques. Even so, that Technical Bulletin does not list all protocols that have seen extensive and satisfactory clinical application.

Among the various options in oxytocin induction administration, the time interval between increases can be varied, and also the rate at which the dosage is increased can be varied independently of whatever time interval is chosen. Several recent reports have compared shorter versus longer time intervals between dosage increases.[3-5] In contrast, we have sought to compare slower versus faster rates of dosage increase for labor induction, with the time intervals approximately the same. Both of our study arms had "longer" intervals between doses, as suggested by Seitchik,[12] because of the time necessary for a given dose to reach its maximum effect. We are unaware of any previous randomized trials making such a comparison.

There are several points to be made concerning the

Table V. Uterine hyperstimulation and FHR patterns of oxytocin induction groups

	Traditional protocol (n = 68)	*Experimental protocol (n = 70)*	*p Value*
Presence of uterine hyperstimulation (No.)			
Yes	41 (60.3%)	44 (62.9%)	0.76
No	27 (39.7%)	26 (37.1%)	
Uterine hyperstimulation resulted in FHR alterations (No.)			
Yes	10 (14.7%)	15 (21.4%)	0.59
No	30 (44.1%)	28 (40.0%)	
No hyperstimulation occurred	28 (41.2%)	27 (38.6%)	

Table VI. Contraction and FHR patterns of oxytocin induction groups

	Traditional protocol (n = 68)	*Experimental protocol (n = 70)*
Contraction pattern (No.)*		
Normal	27	26
≥5 contractions per 10 min	29	32
Prolonged contractions	10	11
Baseline ≥25 min	3	8
Coupling	8	8
Other	0	1†
FHR pattern (No.)‡		
Normal	28	19
Variable	29	34
Late decelerations	3	7
Prolonged decelerations	14	14
Decreased variability	10	10
Other	2	4

*More than one contraction pattern may be reported for some patients.

†One patient experienced dysfunctional labor with no hyperstimulation.

‡More than one FHR pattern may be reported for some patients.

methods. First, the randomization worked quite well, with patient demographics and clinical characteristics similar between the two groups. Second, this study concerned induction of labor, and all patients with indications for induction were evaluated for inclusion into the study. Because there are a multitude of indications, we felt this would be more clinically valuable to the clinician than evaluating only one indication (i.e, postdate pregnancy). Finally, in evaluating temporal data we purposely deleted the failed inductions and cesarean patients. The three patients with failed inductions who were delivered 3 to 10 days later could not plausibly be included in the analysis of temporal data. If all cesarean section patients were included in the data for the induction to regular labor interval, the conclusions would remain the same, with the experimental group attaining labor in a shorter time interval. The inclusion of cesarean section patients in the induction to delivery analysis was not calculated because it would not provide meaningful information to the clinician. The timing of the cesarean delivery was not based on cervical status at the time of the operative procedure and would therefore not be reflective of the actual stage of labor or progression.

Five of our findings are of particular interest. First, patients in the experimental protocol reached a predetermined milestone of uterine activity 1 hour 25 minutes faster than patients in the traditional protocol ($p = 0.03$). It is quite common for patients to require >8 mU/min oxytocin for induction. Hauth et al.[13] demonstrated that 82% of patients required ≥10 mU/min oxytocin to achieve adequate labor. Indeed, in this study the dosage of oxytocin at the time of adequate labor approached 9 mU/min in both groups.

Second, although the experimental group had an induction-delivery interval of 1 hour 6 minutes (1.1 hours) shorter than that of the traditional group (slower increase), this difference did not reach statistical significance. A parallel and perhaps equally important finding is that there is so much variation in the length of labor in both groups in this study that demonstrating a difference would require a randomized trial larger in size than most that have been reported in this field of investigation. Indeed, this trial is larger than most, but because of the large standard deviation of the induction-delivery interval, this group of 138 patients would only be able to detect a difference of 2.5 hours in the induction-delivery interval, with a power of 80%. Without a difference in hyperstimulation risk and with only a small difference in absolute oxytocin dosage between protocols, the more rapid onset of uterine activity in the experimental group tilts these data in favor of that protocol, pending a more definitive answer to this question. It seems plausible to us that once adequate labor is established, so long as hyperstimulation and other problems do not ocur, it doesn't matter which protocol is used.

Third, safety issues are primarily related to hyperstimulation, which was equivalent with these protocols in our hands. Although our rates of hyperstimulation have precedent,[14] our definition was particularly strict because of our desire to determine if one protocol was harder to control than the other. Also, although there

Table VII. Oxytocin management

	Traditional protocol (n = 68)	*Experimental protocol (n = 70)*	*p Value*
Maximum oxytocin dosage for patient (mU/min, mean ± SD)	11.34 ± 5.88	13.39 ± 7.10	0.07
Dosage of oxytocin at time of adequate labor* (mU/min, mean ± SD)	8.33 ± 5.19	8.90 ± 6.70	0.60
Oxytocin management modified for uterine hyperstimulation or FHR changes (No.)			
No changes	37 (51.6%)	29 (40.6%)	0.27
Decreased	11 (17.7%)	12 (17.4%)	
Discontinued temporarily	20 (30.7%)	29 (42.0%)	

*Thirteen women did not achieve adequate labor and were not included in this calculation.

Table VIII. Labor patterns for vaginal deliveries

	Traditional protocol (n = 53)	*Experimental protocol (n = 53)*	*p Value*
Induction to establishment of regular labor pattern (hr, mean ± SD)	4.85 ± 4.22	3.43 ± 2.15	0.03
Induction to delivery (hr, mean ± SD)	10.04 ± 5.50	8.98 ± 5.60	0.33
Rupture of membranes to delivery (hr, mean ± SD)	11.59 ± 9.01	11.36 ± 8.32	0.89
Epidural anesthesia to delivery (hr, mean ± SD)	5.69 ± 4.00	6.80 ± 5.68	0.38

was an increase in the maximum oxytocin dose in the experimental group, we do not believe this very small increase is clinically significant.

Fourth, FHR alterations versus normal heart rate patterns were noted to have occurred more frequently in the experimental group, although this was not statistically significant. This can be concerning. Variable decelerations occurred frequently and more often in the experimental group. Late decelerations occurred infrequently in both groups, although there was a small increased incidence in the experimental group. In analyzing these results, we found that the two groups did not differ in the incidence of uterine hyperstimulation resulting in FHR alterations (Table V). The data do not support that the variable or late decelerations were related to hyperstimulation and thus the oxytocin protocol. Therefore it is not clear that the safety of induction was altered by the oxytocin protocol. Of importance, the perinatal outcome was similar between the two groups. Nonetheless, the alteration in FHR, if affiliated with the oxytocin protocol, may outweigh any benefits an early labor pattern may bring.

Finally, there were more operative vaginal deliveries in the experimental group. This was initially a surprise finding. Akoury et al.,[15] however, have also suggested a higher incidence of operative vaginal deliveries in an aggressive oxytocin protocol. On review of our fetal monitor strips, none of these patients had operative vaginal deliveries secondary to hyperstimulation-related FHR changes. An explanation for this finding, if not related to hyperstimulation or to chance, is not straightforward. Possibly an intrinsic factor pertaining to the oxytocin protocol allows for more episodes of second-stage cord compression and thus more operative vaginal deliveries. This factor could be of concern, and obviously more study is required before a full explanation is found.

Several recent additions to the oxytocin literature have advanced our knowledge and understanding of this drug, which is so uniquely identified with obstetric practice. Mercer et al.[5] reported a randomized trial of 123 patients in two study arms, both of which used dosage increments similar to our experimental group. A longer time interval group (≥60 minutes) has less hyperstimulation and fewer cesarean sections for fetal distress than did a 20-minute time increment group. Their data suggest that longer time intervals produce less hyperstimulation, consistent with Seitchik's and Castillo's[14] finding that a longer time interval between dose increases was associated with less hyperstimulation.

Unlike Mercer et al., we found no difference in hyperstimulation between our study arms, perhaps because of a longer time interval in both of our groups. Interestingly, Mercer et al. had induction-to-delivery standard deviations even larger than ours. Their standard deviations for this interval of 8 hours (SEM 1.1 with 53 patients) and 7.8 hours (SEM 1.2 with 43 patients) were similar to ours.

Blakemore et al.[4] randomized 52 patients into 15-minute and 60-minute arms with mixed types of dosage increases. The hourly group received less oxytocin, but

overall effectiveness of the regimens was uncertain, with standard deviations of 7.85 and 7.4 hours in the two groups and a power of 0.8 to detect a 55% difference in the length of labor.

In conclusion, several authors have supported the concept that slow rate of increase of oxytocin administration may be advantageous in the induction of labor. Our study demonstrates that larger increments in association with slow intervals may improve efficacy by obtaining labor sooner without altering incidence of hyperstimulation or perinatal outcome. However, the difference in FHR changes and operative vaginal deliveries suggest caution in concluding that the large incremental protocol is clinically preferable until more data are available.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

DR. TERRY B. LEVENSON, Conway, South Carolina. In 1906 Dale demonstrated that myometrial tissue would contract when exposed to posterior pituitary extracts in vitro. By 1913 Watson had begun to use such extracts for the induction of labor. Complications such as uterine rupture and its attendant morbidity and mortality discouraged this practice until Theobald et al. described the administration of drip oxytocin in 1948. In 1949 DuVigneaud demonstrated the structure of the octapeptide oxytocin, work for which he was later awarded the Nobel Prize in Chemistry. Synthetic production of oxytocin soon followed.[1]

Possession of this powerful mediator of uterine contractility begs the question what is the safest and most effective way to use it?

To this end a number of studies have been done to demonstrate that this medication can be used in variety of clinical stiuations, but comparative studies of various protocols have been few. The article presented by Muller et al. compares the effectiveness of two protocols for administering oxytocin to induce labor.

The control and experimental groups in their study were remarkably similar in terms of patient demographics and indications for induction of labor. Bishop scores, cervical dilatation at the onset of induction, and use of prostaglandin gels and epidural anesthesia were likewise very similar.

The control group was given oxytocin at 1 to 2 mU/min, and the infusion was increased by 1 to 2 mU/min at 30-minute intervals until adequate labor occurred. The experimental group received the same starting dose, but infusion rates were doubled every 40 minutes until a rate of 8 mU/min was reached, and then increases were made in smaller increments to advoid hyperstimulation of the uterus.

Maximum doses of oxytocin tended to be higher in the experimental group, but this difference was not statistically significant. Dosages infused at the time adequate labor was achieved were similar in both arms of the study.

Hyperstimulation occurred in both groups with equal frequency but did not lead to untoward outcomes. Management difficulties were similar in both groups in that decreasing the infusion rate or discontinuing the infusion temporarily was required as often in the experimental group as in the control group.

Abnormal FHR patterns were seen more often in the experimental group. This was believed to be due to a higher incidence of variable decelerations in this group. Careful analysis failed to show a relationship between hyperstimulation and these decelerations, indicating this effect was not due to the ocytocin infusion protocol.

Cesarean section delivery occurred with equal frequency in both groups. Fetal distress was the indication

in only two of 62 patients in the control group and three of 69 in the experimental group. Dystocia was the most common indication, occurring with equal frequency in both groups.

More assisted vaginal deliveries occurred in the experimental group than the control group, most of which were for FHR changes in the second stage of labor. These were not believed to be due to uterine hyperstimulation.

Obstetric outcomes were uniformly good in both groups, and no infants delivered had 5-minute Apgar scores <7.

The experimental group achieved labor an average of one hour and 28 minutes faster than the control group, a significant difference. They were delivered 1 hour 12 minutes sooner than controls, but this differential failed to reach statistical significance.

Muller et al. have demonstrated that oxytocin infusion rates for the induction of labor can be safely increased by relatively large increments, provided adequate time is allowed for the maximal contractile response to occur. Fuchs et al.[2] have shown that plasma levels of oxytocin in patients receiving up to 3 mU/min are not significantly different from levels before the induction of labor. In their study plasma levels seen in patients in spontaneous labor were not reached until infusion rates reached 4 to 6 mU/min. Blood levels of oxytocin then increased linearly in relation to time and infusion rate.

By doubling the infusion rate at low dosage levels, Muller et al. have apparently demonstrated a method of reaching effective plasma concentrations of oxytocin in a shorter amount of time than traditional protocols allow.

I would like to ask Dr. Muller to comment on two points. First, the incidence of abnormal FHR changes seems quite high in both arms of the study. Your analysis suggests that epidural anesthesia is a major factor in these events. Are your patients who receive epidural anesthesia given preloads of intravenous crystaloid to prevent the hypotension that may accompany the onset of anesthesia and lead to placental underperfusion and subsequent FHR alterations? Second, can you cite any figures regarding the incidence of abnormal FHR changes in unstimulated labor?

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DR. MULLER (Closing). Some of the literature suggests that expanding the time interval between increasing oxytocin doses gives a safer, and just as effective, method of induction. Our purpose in this study was to see whether we could get to adequate levels sooner by increasing the increment dosage while at the same time still using longer intervals. Specifically, we studied whether we could decrease the number of induction days or decrease the time to delivery by getting up to a higher oxytocin level sooner than in the ACOG protocol.

In answering Dr. Levenson's questions regarding epidural usage, the number of epidural anesthetics given were equal in both groups, as I noted earlier. They were placed by the residents on staff at Carolinas Medical Center in a similar manner. All patients were given 1 L of intravenous saline solution before the anesthetic was administered. I did not, however, collect data on patients as to whether complication such as hypotension occurred.

We did find a high rate of FHR alterations. This finding is of concern. However, we defined FHR alterations very strictly. If a patient had one episode of variable deceleration <100 beats/min, we labeled that patient as having had FHR alterations.

I do not have data available for the percentage of unstimulated labors with FHR alterations. Several studies exist that have compared the aggressive use of oxytocin versus not using oxytocin for augmentation with the percentages under 50%, whereas ours were all over 50%.

Further study has to be done concerning assisted vaginal deliveries. Some of the Canadian literature[1] also suggests that aggressive forms of oxytocin administration result in an increased incidence of assisted vaginal deliveries. At this point I do not have an explanation for our increased incidence other than that it does not seem to be related to hyperstimulation nor does it seem to be related to the oxytocin protocol.

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The use of carbon dioxide laser laparoscopy in the treatment of tubal ectopic pregnancies

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OBJECTIVE: The purpose of this study was to assess the efficacy of the treatment of unruptured tubal ectopic pregnancies by the use of carbon dioxide laser laparoscopy.
STUDY DESIGN: A series of 125 consecutive ectopic pregnancies were treated laparoscopically; the tubal pregnancy was removed by a laparoscopic laser technique. Preoperative assessment included monitoring β-human chorionic gonadotropin levels, use of vaginal ultrasonography, and preoperative and postoperative hematocrit levels.
RESULTS: Laparoscopic laser surgery was successful for removal of tubal ectopic pregnancies in all but four patients, in whom a laparotomy was required. Hematocrit levels before and after surgery were similar. The time necessary for β-human chorionic gonadotropin to fall to nondetectable levels averaged between 3 and 4 weeks. There were five patients who had complications requiring additional surgery and/or medical treatment.
CONCLUSION: The techniques are easy to learn, and the use of laparoscopic laser surgery in the treatment of tubal ectopic pregnancies appears to be a safe procedure with definite advantages for both the patient and the physician. There are decreased operating times, shorter hospital stays, and lower medical costs compared wtih those for major surgery. Subsequent successful intrauterine pregnancy rates are comparable to those of conservative methods previously reported. (Am J Obstet Gynecol 1992;167:382-6.)

Key words: Laser, laparoscopy, ectopic pregnancy

Conservative surgery in the management of tubal ectopic pregnancies was uncommon during the early part of this century. There were several series in the German literature in the early 1940s[1, 2] describing reconstructive procedures. Some patients subsequently had intrauterine pregnancies. The first reference in the English literature was that by Stromme.[3] He described a conservative surgical procedure for the treatment of an unruptured tubal pregnancy in his patient's only remaining tube. The patient subsequently had two intrauterine gestations. In 1973 Stromme[4] published a 20-year review of his experience with these procedures. His technique was virtually unchanged during that time frame; only his choice of suture was different. The technique of linear salpingostomy has changed appreciably since that time because sutures have become less reactive, less traumatic forms of tissue incision have been implemented, and microsurgical technique has been used. It has become medically accepted that conservative management of a tubal pregnancy should be considered in any patient in the reproductive age group desiring further pregnancies. This surgery should be considered even if the opposite tube appears normal, because studies have demonstrated that either tube has the same chance of having a repeat ectopic pregnancy.[5] It has also been demonstrated that in women wishing to preserve their fertility the rates of recurrent ectopic pregnancy and intrauterine pregnancy after conservative management seem to be equivalent or superior to the results after radical treatment with salpingectomy.[6] These conservative methods were originally all performed during a laparotomy. In the early 1980s reports of laparoscopic removal of ectopic pregnancies began to proliferate.[7-9] Their procedures involved making an incision on the antimesenteric border of the tube overlying the ectopic pregnancy, controlling hemostasis with careful electrocoagulation and allowing secondary closure of the tube. Patency, subsequent fertility potential, and recurrent ectopic pregnancy rates were comparable with those procedures performed during laparotomy. As the methods and techniques in reproductive surgery have increased, the laser has become an important tool. Its use in laparoscopy has made it a valuable aid in the removal of ectopic pregnancies. A series of 125 consecutive patients with tubal ectopic pregnancies, primarily treated by laser laparoscopy, is presented.

From the Institute for Reproductive Medicine.
Presented as Official Guest at the Fifty-fourth Annual Meeting of The South Atlantic Association of Obstetricians and Gynecologists, Palm Beach, Florida, January 26-29, 1992.
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6/6/38065

Material and methods

Patient profile. One hundred twenty-five consecutive patients seen at the Institute for Reproductive Medicine

with ectopic pregnancy were included in this study. The average age was 36.4 years (range 26 to 40 years). The patients were fairly equally divided between primary (55 patients) and secondary (68 patients) infertility. The average duration of infertility was 2.85 years (range 1 to 7 years). Two patients did not have an infertility problem. Preoperative and postoperative hematocrits were determined, as were preoperative and postoperative β-human chorionic gonadotropin (β-hCG) levels. One hundred twenty patients had ectopic pregnancy in the ampullary portion of the tube, whereas five patients had isthmic tubal ectopic pregnancies.

Diagnosis of ectopic pregnancy. Patients undergo follow-up with β-hCG results when the diagnosis of pregnancy is established. It is expected that the β-hCG level will follow a curve that demonstrates a doubling in value every 2 to 3 days. If the level does not remain on this "normal" curve, it is assumed that the pregnancy is not normal. If the level is >1500 mIU/ml, a transvaginal sonogram can be obtained. If a gestational sac is seen, then for all intents and purposes an ectopic pregnancy is ruled out. If no sac is noted in the cavity of the uterus, then there is a high level of suspicion for ectopic pregnancy. If the β-hCG level does not continue a normal doubling and the level is >1500 mIU/ml, a curettage can be performed when there is suspicion of an ectopic pregnancy, and tissue can be sent for frozen section. If no chorionic villi are seen, a laparoscopy can be performed with the presumptive diagnosis of ectopic pregnancy.

Technique. The surgical approach to laparoscopic removal of ectopic pregnancy is one that involves a three-puncture technique. A laser laparoscope, which includes an operative-laser channel, is placed through an infraumbilical incision. The articulated arm of a carbon dioxide laser is connected to the channel allowing the laser beam to be delivered to the tissue. A 5 mm second puncture probe (suction and irrigation) is placed through a midline suprapubic incision. A third incision for the grasping forceps is made lateral to the rectus muscles at the level of the anterior external iliac spine on the ipsolateral side of the tubal pregnancy. All procedures are videotaped with a television camera that has a beam splitter coupled to the eyepiece of the laparoscope while the physician uses a video camera and works from the monitor. The size and site of the tubal pregnancy are identified, the mobility of the tube is assessed, and, if feasible, the procedure is carried out by first infiltrating a dilute solution of vasopressin (20 U in 40 to 50 ml normal saline solution) into the antimesenteric border or into the mesosalpinx of the tube in the area adjacent to the ectopic pregnancy to minimize bleeding from small vessels. If the size of the ectopic pregnancy is >6 cm in length and 3 to 4 cm in diameter, the expectation for increased bleeding is increased and it may prove more prudent to perform the procedure by laparotomy. The tube is then stabilized with the grasping forceps, and with the carbon dioxide laser beam through the operating channel of the laparoscope, an incision is made on the antimesenteric border over the area of the ectopic pregnancy. The power used is between 15 to 25 W in a superpulsatile mode with a spot size of 0.5 mm. Grasping forceps are then placed through the operating channel of the laparoscope after the coupling device of the laser is removed, and the tissue is gently teased out of the tube and pulled out through the operating channel. The bed is then inspected. If there is any bleeding, especially in the area of the tube where the incision was made, it can be coagulated with a bipolar cautery or with a defocused laser beam. Usually the tube has very little bleeding with this procedure. After hemostasis is secured, the instruments are removed and the patient can be brought to the recovery room and be discharged within 3 to 12 hours.

Results

Of the 125 consecutive patients seen with an ectopic pregnancy in the fallopian tube, four patients required laparotomy. One of the patients had a tubal pregnancy greater than 10 × 5 cm. It was elected during laparoscopy, on seeing the size of the ectopic pregnancy, to immediately perform a laparotomy for repair. A second patient had an ectopic tubal size of 6 × 3 cm. The ectopic pregnancy was successfully removed, but after a faulty bipolar cautery and continuous manipulation while trying to cauterize the oozing point, increased bleeding necessitated a laparotomy for "one stitch." The third and fourth patients had inaccessible tubes because of excessive adhesion formation.

The remaining patients had a benign postoperative course after laparoscopy. The preoperative hematocrit (37.2% ± 1.8%) was similar to that postoperatively (35.5% ± 2.8%). The patients left the outpatient surgical center or hospital 3 to 12 hours after the procedure. Their recuperation and ability to perform their normal everyday functions were similar to those of patients having laparoscopies for other indications. There were five patients in whom the serum β-hCG level did not continue to drop. In all these patients the levels of pregnancy hormone initially fell and within 1 week had risen, often to a level greater than that before operation. Two of these individuals had a partial salpingectomy. The remaining three patients had methotrexate therapy. With the exception of these five patients, the postoperative serum β-hCG levels continued to drop to <10 mIU/ml after an average duration of 21 to 28 days.

Tubal patency was determined in each patient 4 to 16 weeks after operation. With those patients who had laparotomies or additional treatments excluded, hysterosalpingography was performed in the majority of patients (71 patients), and a second-look laparoscopy was performed in 45 patients. Nonpatency of the in-

volved tube was noted in only two of the patients undergoing hysterosalpingography. Patency was seen in all patients who had laparoscopy; however, there were two patients who did not have total healing of the fallopian tube and in whom a rent or "hole" remained with tubal mucosa protruding out. Several patients also had granuloma-like areas in the affected tubal areas associated with tubal patency.

Forty-eight patients have subsequently attempted for at least one year to become pregnant. Thirty-eight have been successful. Of these patients, 26 have had intrauterine pregnancies and 12 have had repeat ectopic pregnancies, seven in the same tube and five within the contralateral tube.

Comment

The carbon dioxide laser for the treatment of tubal ectopic pregnancies has been used since the mid-1980s and was first reported in a small series by Johns and Hardie[10] in 1986. Although the tubal incision can be performed over the ectopic pregnancy by laser, scissors, or electrocautery, the laser holds several distinct and theoretic advantages over the other modalities. It is more hemostatic than scissors and there is less tissue reaction compared with electrosurgery in animal studies. The procedure is simple, quick, and easy to learn and perform. Other pathologic conditions within the pelvis can be easily addressed surgically and complications are uncommon.

Within this series there was no increased blood loss during or after the procedure. The time required for the laparoscopic procedure ranged from 20 minutes to just over 50 minutes of operating time. Hospitalization occurred only as a help to patients if the procedure was performed in the evening, to allow the patient to rest in a hospital bed, because the outpatient facilities in the hospital are not functioning at night. When done as an outpatient procedure, these procedures obviously decrease medical costs in comparison with laparotomy with its inherent hospitalization costs. The ability of the patient to return to normal function, whether at work or at home, allows for a diminished loss to the patient, family, or work area.

The management of tubal pregnancy by the laparoscopic approach offers most of the advantages of laparotomy without its disadvantages. A reduced morbidity and cost associated with equal or better subsequent reproductive outcome in these patients warrants a movement toward laparoscopy over laparotomy if surgically feasible. Laparoscopic laser salpingostomy as a treatment for tubal ectopic pregnancies appears to be a safe procedure with many advantages for the physician and patient. As a hemostatic procedure with good reproductive outcome for the patient, this surgical procedure offers itself as a possibility for reparative treatment of tubal pregnancies. As a procedure associated with decreased operating time, diminished hospital stay, and reduced medical costs in comparison with major surgery, laser laparoscopy is becoming a logical choice over laparotomy as a treatment for unruptured tubal ectopic pregnancies.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

Dr. W.E. Gibbons, Norfolk, Virginia. Dr. Paulson's thoughtfully prepared manuscript on laparoscopic treatment of ectopic pregnancy with the carbon dioxide laser represents one of the largest reported experiences in this area. He correctly documents the shift in therapy of this disorder from laparotomy and salpingectomy to linear salpingostomy by laparoscopy. The approach to diagnosis and surgical management were straightforward and conventional. Initial laparoscopic approach to management was achieved in all except four patients and in only one case was it a method failure due to inability to control bleeding. The carbon dioxide laparoscopic management as complete therapy was unsuccessful in only five women in whom postoperative, increasing hCG titers were observed (5/121, 4%). This demonstrates a very successful treatment outcome for an outpatient, surgical therapy where the options are either a major surgical procedure with its associated increased morbidity or protracted medical therapy, which has a similar failure rate and its own set of complications.

An important component of this manuscript is the

follow-up data. Only two of 71, or 3% of subjects undergoing hysterosalpingographic evaluation had tubal blockage demonstrated in the treated tube. Of the 45 subjects undergoing second-look laparoscopy, all of the treated tubes were patent. A question for Dr. Paulson concerns how the decision was made to perform second-look laparoscopy as to why some underwent the procedure and others did not and as to how the timing of the follow-up procedures (4 to 16 weeks) was arrived at. It would be interesting to have information from the second-look laparoscopies concerning new adnexal adhesion formation resulting from the earlier treatment laparoscopy and how often lysis of adhesions was performed during the second-look procedure. The subsequent intrauterine pregnancy rate and ectopic pregnancy rate are similar to those of other series reported in the literature.[1, 2] Was information gained at the second-look laparoscopy useful in predicting who would ultimately conceive? Would life-table analysis of this information be productive? The publication of the hCG disappearance curve will prove useful to others who appropriately follow the hCG titer after conservative surgery.

Recently, Vermesh and Presser[3] reported on 60 women with ectopic pregnancy undergoing linear salpingostomy who were randomized between laparotomy and laparoscopy. Although even though there was no difference observed in the subsequent viable pregnancy rate at 36 months (laparoscopy 12/19, 63%; laparotomy 11/21, 52%), life-table analysis demonstrated that the laparoscopy patients conceived earlier. Lundorff et al.[4] reported last year that in 73 of 105 ectopic pregnancy patients treated with conservative surgery by either laparotomy or laparoscopy who underwent second-look laparoscopy the laparotomy patients developed significantly more adhesions in the ipsilateral adnexa than did the laparoscopy patients. These data support Dr. Paulson's assertion of the desirability of the laparoscopic technique in the hands of a trained laparoscopist.

Stoval et al.[5] reporting on the largest experience with methotrexate therapy in this country recently reported a similar method failure rate (4/100) and similar follow-up rates for tubal patency hysterosalpingogram, 85%) and percentage of subsequent ectopic pregnancies (11%). Perhaps in the future medical therapy will be the standard of therapy. In my opinion, until the experiential base with medical therapies markedly increases, laparoscopic, outpatient management with its clear end point is still the therapy of choice with medical therapy reserved for treatment failures, the ectopic pregnancy whose location cannot be determined, and the rare condition of disseminated peritoneal trophoblastic implants.

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DR. FRANK MCDONALD, Hickory, North Carolina. I was interested in the five patients with treatment failure. You mentioned that you got frozen sections for villi from curettage reports. I wonder, if you had frozen sections from those five ectopics, whether there was truly ectopic tissue and villi there and whether you would consider frozen sections for your ectopic tissue in the future.

DR. JOHN SCOTT, Spartanburg, South Carolina. Dr. Paulson, I have several questions. Did you use serum progesterone values at all? When you made your linear incision did you use a superpulse mode or standard wattage and, if you did the latter, what wattage did you use? How did you remove the larger ectopic pregnancies from the abdomen? Did you ever have to do a colpotomy and remove it vaginally?

DR. WILLETTE L. LEHEW, Norfolk, Virginia. I would like to ask Dr. Paulson what, if any, indications he would use to close the tube with suture?

DR. PAULSON (Closing). I use the superpulsatile mode because it will cut through the edematous tissue that has also been injected with dilute vasopressin solution. If you use continuous 25 to 30 watts you get a lot of smoke, whereas superpulse, which can pulse from 250 to 500 watts as a small part of the duty cycle, cuts through very nicely.

Usually the ectopic pregnancy is removed from the abdomen piecemeal. You may have to do it by taking the whole scope out of the largest trocar sheath. You usually do not have to peform a colpotomy.

Normally I do not use suturing techniques. We might use suturing for hemostatic purposes if there was bleeding, but this is usually not a problem because we pick up our ectopic pregnancies very early in their course.

We did not get frozen sections on our ectopic tissue at the time of surgery. All specimens were subsequently evaluated and did show villi at least in this particular series.

I do not know how many patients in this particular series had a previous ectopic pregnancy. We have done many procedures since this series, and we have had repeats on some of them. We did not do life-table analysis. It would have been useful. We did find adhesion formation in the second-look laparoscopies but not in a large number. Every once in a while there would be adhesions on the tube that were usually very simple just to peel off. As far as predicting future pregnancies, I

cannot predict that even those with little rents in the tube won't get pregnant. I think we have women with intrauterine pregnancies even when they have something like that. At the time of a second-look laparoscopy, I might try to create a raw area over such a rent in the tube so it will reperitonealize.

I was asked why we did second-look laparoscopies instead of hysterosalpingography on some of our patients. It was mostly patient choice. I am very aggressive and I feel that second-look laparoscopies are helpful because it will add to future fertility and predictive values. However, the majority of our patients did not have second-look laparoscopies and we have not analyzed our data on second-look laparoscopy versus hysterosalpingography, so I don't know how predictive it would be.

Intrauterine pressure and fluid absorption during continuous flow hysteroscopy

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OBJECTIVES: Our objectives were to document the causes of fluid absorption during continuous flow hysteroscopy and to determine under which operative conditions fluid overload may occur.
STUDY DESIGN: Fifteen patients underwent operative hysteroscopy with 2% ethanol solution for uterine distention. Absorption of fluid was measured by blood alcohol, sodium, osmolarity, and hematocrit. Intrauterine pressures were measured with an obstetric pressure catheter.
RESULTS: Alcohol absorption was noted in one patient during a myoma resection. Two additional patients, not in the study, had fluid absorption after partial perforations of the uterus. Under normal operative conditions there were no changes in sodium, osmolarity, or hematocrit. Intrauterine pressures ranged from 45 to 75 mm Hg. Experimental pressures of >200 mm Hg were not associated with fluid absorption.
CONCLUSIONS: Intravasation of fluid may occur through open uterine venous channels with extensive resections and under low pressures in the presence of unrecognized perforations. (Am J Obstet Gynecol 1992;167:386-91.)

Key words: Hysteroscopy, fluid absorption, intrauterine pressure, ethanol, partial perforation

Diagnostic and operative hysteroscopy procedures are done in a variety of settings with different distention media. The use of continuous flow distention with low-viscosity fluids such as 5% dextrose in water, saline solution, and glycine are popular for intrauterine laser, resections, coagulation, and ablations. However, there is a risk of irrigation fluid absorption leading to volume overload and a rare risk of death subsequent to pulmonary edema or hyponatremia.[1] The mechanism of this intravasation is poorly understood but appears to be similar to complications of urologic transurethral prostate resection.

The objective of this study is to measure some of the mechanisms involved in systemic absorption of irrigation fluid and to determine under which operative conditions volume overload can occur.

Methods

Fifteen study participants were chosen from patients scheduled for diagnostic and operative hysteroscopy; they gave their full informed consent as approved by the Committee on the Protection of Human Subjects

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at the University of North Carolina Hospitals. Uterotubal cannulations, endometrial ablations, myoma resections, and septum resections were the indications for the procedures, which were mostly carried out with the patients under general inhalation anesthesia in the proliferative phase with no hormonal preparation. Pitressin was used during ablations and myoma resections only. A 24F Wolf resectoscope was used, and for myomas and resections the cervix was overdilated to 37F.

Glycine, a nonelectrolyte solution commonly used for transurethral prostate resection by urologists, was used as an irrigant. Ethanol was added to create a 2% solution. Fluid absorption was then measured directly by blood alcohol levels. In addition, indirect estimates of fluid absorption were made by measuring the volume of outflow collected and by subtracting this volume from the amount of irrigant (inflow) used. Also, calculations of intravascular fluid absorption were made by measuring plasma sodium, osmolarity, and blood hematocrit.

Blood samples were obtained and pressures were measured at the following times during a procedure:

Time 1 Beginning of the procedure (baseline)
Time 2 After 5 minutes with 8 feet of pump pressure
Time 3 After 5 minutes with 14 feet of pump pressure or at 8 feet with outflow restricted
Time 4 End of procedure

Intrauterine pressures were measured directly with a standard obstetric pressure catheter placed alongside the hysteroscope inside the uterine cavity.

A uterine model was also created for laboratory measures of flow rates and pressures with a variety of pump settings, pump types, and different hysteroscopes. The patients were used as their own controls, and one-way analysis of variance statistical measures were used to compare changes in plasma sodium, osmolarity, and blood hematocrit with a significance level at $p < 0.05$.

Results

In vitro studies. The laboratory model used (Fig. 1) documented that a number of independent variables influenced intrauterine pressure and flow rates. These included the following: (1) height of fluid column or pump setting, (2) manufacturer of hysteroscope, (3) degree of cervical dilation, and (4) outflow valve closed or open. We first calibrated the pressure reading of our uterine model with a closed system (no cervical or valve outflow) in an operating room, simulating conditions of surgery. The three points measured were at 4, 6, and 8 feet of direct gravity flow, resulting in intrauterine pressures of 118, 177, and 230 mm Hg, respectively. Higher direct gravity measures were not possible, because the operating room ceilings are not higher than 8 feet from the operating table surface. The Bard Hystero-Flo (C. R. Bard, Tewksbury, Mass.) pump matched this calibration curve at 8 and 14 feet of pressure.

We next measured the effect of opening the outflow valve and simulating cervical overdilation by allowing fluid to flow around the hysteroscope. Opening the outflow valve decreased pressure to about 50 to 60 mm Hg, and opening the cervix further decreased pressures to 40 to 50 mm Hg. Under these conditions there was minimal difference in intrauterine pressure with Bard pump settings of 8 or 14 feet of pump pressure.

Urologists routinely use the outflow valve to regulate flow rates and pressures. Using the outflow valve to maintain the intrauterine pressure at either 50 or 80 mm Hg (Fig. 2), there was only a 12% difference in flow rates with Bard pump settings of 8 or 14 feet, and flow was approximately 375 to 450 ml/min.

The Zimmer pump is designed with an upper limit of 80 mm of pressure, with a readout measured at the inflow valve (approximating intrauterine pressure). When 80 mm of measured intrauterine pressure was maintained, flow rates were reduced, even at the highest pump setting, to 150 ml/min (Fig. 2).

Fig. 3 shows intrauterine pressure and flow rates with two different hysteroscopes. Surprisingly, the larger (24F) Wolf resectoscope had lower flow rates and higher resistant pressures than the smaller (22F) Olympus pediatric resectoscope.

Clinical studies. Intrauterine pressure data from 14 patients thus far show a range of 45 to 75 mm Hg (with an average 63 mm Hg) under normal operative conditions (open cervix or outflow valve, no perforations). Closing overdilated cervices with a tenaculum for 5-minute periods raised intrauterine pressure to an average of 122 mm Hg. In some patients, both the cervix and outflow valve were closed, creating intrauterine pressures of 230 and 280 mm Hg with 8 and 14 feet of pump pressure, respectively. No fluid absorption as measured by blood alcohol, volumetric discrepancy, or hyponatremia was seen during these in vivo pressure experiments.

Blood studies on 15 patients revealed nonsignificant average declines in serum sodium from 139 to 136 (time 1 and time 4), $p > 0.05$. Average osmolarity was 285 to 289, and the mean hematocrits were 35 to 33. The mean measure of volumetric fluid deficit was 152 ml.

One patient out of 15 had ethanol detected (37 mg/dl) at the end of the procedure. This patient had had a myoma resection and both the cervix and outflow valve had been closed for 3 minutes to increase visualization due to excessive bleeding. Through an oversight the valve had remained closed for nearly 10 more minutes. The pressure catheter was not functioning in this case. The volumetric measurement of absorption in this case was 900 ml, equivalent to the estimated volume required to reach a blood level of 37 mg/dl

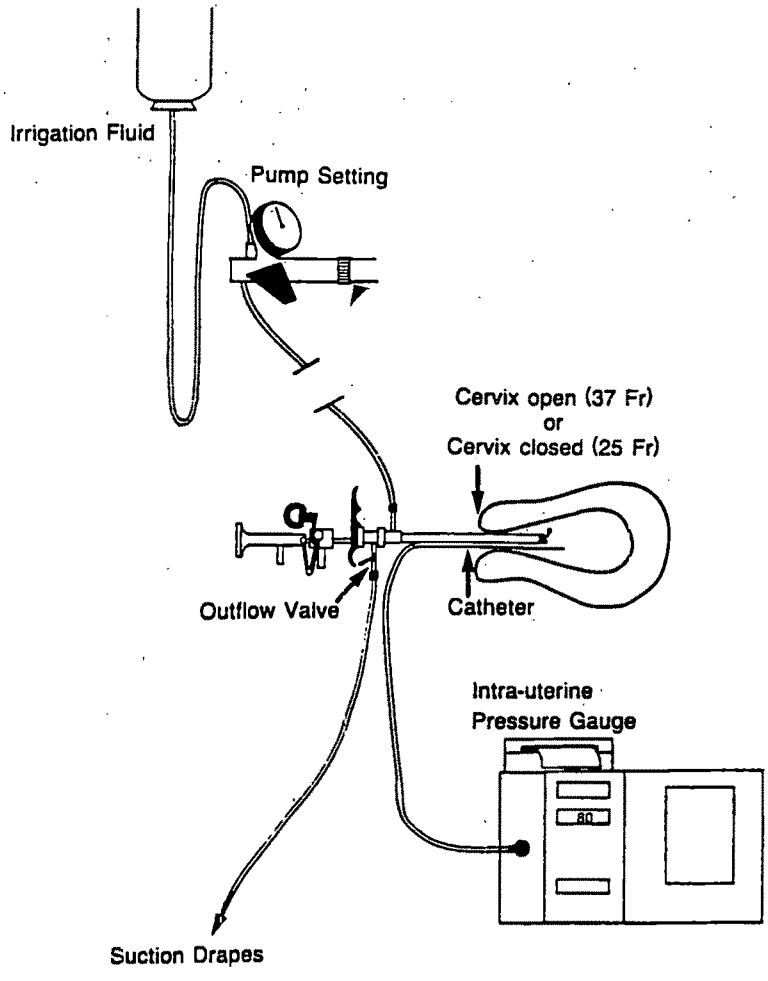


Fig. 1. Experimental model. Variables of fluid dynamics include pump settings and types, type of hysteroscope, cervical dilation, outflow valve.

ethanol, given a 5 L blood volume and 2% ethanol solution.

One other patient, not in the study, developed a lateral tear of the cervix after fibroid removal. The standard pressure settings, estimated at 75 mm Hg, were used for a 3-minute period to diagnose the perforation. During this time the patient had absorbed 1500 ml of fluid by volumetric estimation. This patient did not have any clinical signs of volume overload.

A third patient, not in the study because of severe diabetes and chronic renal insufficiency, developed acute pulmonary edema after a rapid (12 minute) endometrial ablation under local anesthesia. Serum sodium level was 129 mEq/L. There was an absorption, estimated by measuring input and output of the irrigation system, of 1300 ml. There was a suspected thinning and possible partial perforation of the fundus during the dilatation of the cervix during this procedure. There was good outflow from the cervix in this case, allowing an estimate of intrauterine pressure of 60 to 75 mm Hg.

Comment

Hysteroscopic surgery with low-viscosity, continuous-flow irrigation offers several advantages over other systems.[2, 3] These include better exposure in operative cases, continuous clearing of blood and tissue, and fewer reported complications.[4] Nevertheless, the risk of fluid overload and hyponatremia exists and is analogous to the complications with prostatic resection procedures.

The finding of minimal to no absorption during hysteroscopy detected in most of these patients to date is encouraging. Hysteroscopy should be expected to have less than the 5% to 10% incidence during prostatic resection by the urologists.[1, 5] This is due to the fact that prostatic tissue has an extensive plexus of venous sinuses that are opened up during resections, although paracervical venous plexi can be exposed during unsuspected cervical tears.[6]

We have drawn from studies in the urology literature that use ethanol added to the irrigation fluid to monitor absorption during prostatic resection.[7] Hahn[8] in 1988

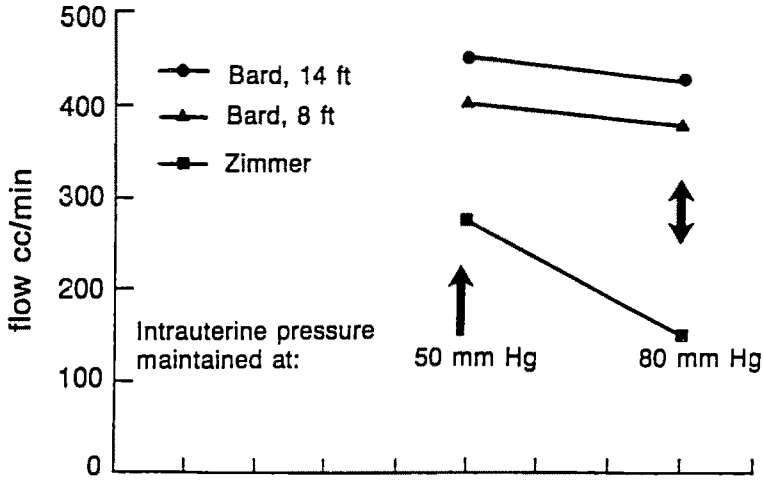


Fig. 2. Factors affecting flow rates (in vitro). Maintaining intrauterine pressures with outflow valve.

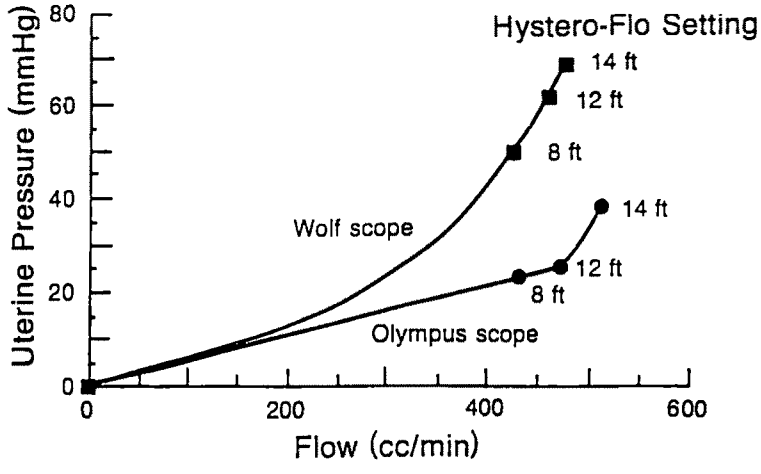


Fig. 3. Uterine pressure versus flow. In vitro comparison of hysteroscopes.

demonstrated that absorbed glycine levels are in direct proportion to serum ethanol levels, whereas there was a proportional decline in the serum sodium levels during prostatic resections. Norlen[9] determined fluid absorption on the basis of the osmolarity of the irrigation fluid (glycine) and the change in serum sodium seen postoperatively. The availability of inexpensive breath-alyzer units may make it possible to routinely monitor the expiratory gases continuously during surgery with a 1% or 2% ethanol irrigant, to detect fluid absorption as it occurs during surgery.[8]

In addition to case reports,[10, 11] Morrison et al.[12] have the only other study in the gynecology literature attempting to measure fluid absorption during hysteroscopic surgery. In one patient of the 12 in the study fluid overload developed after laser ablation procedure in which saline solution was the irrigation fluid. Measurements of absorption show a rise in central venous pressure and serum chloride while the plasma albumin and hematocrit decreased. Intrauterine pressures were not included in the study.

The urology patient tends to be older and more susceptible to volume shifts. Another interesting difference between our patients and the urology patients is gender. In 1986 Arieff[13] reported 16 postoperative deaths over a 10-year period in healthy women undergoing elective general and gynecologic surgery. They were found to have had fluid overload leading to the syndrome of inappropriate antidiuretic hormone resulting in severe hyponatremia. Fraser et al.[14] also reports similar findings in 11 deaths that he attributes to central diabetes mellitus and diabetes insipidus secondary to untreated severe hyponatremia. Fraser and Arieff[15] have also shown in rat studies that the Na^+-K^+ adenosine 5'triphosphatase pump system is less effective in female than in male brain synapses. Progesterone can also decrease pump activity in myocardial and kidney tissue.[13] These studies suggest that guarding against iatrogenic hyponatremia may be even more critical in female patients. However, animal studies in our laboratories have failed to confirm these hypotheses to date.

This is a preliminary report of our studies so far, presented to report some clinically useful unexpected findings. Different pump settings and pumps (Figs. 2 and 3), result in wide differences in pressures and flow rates, as do different hysteroscopes (Fig. 3). Contrary to the current assumption that elevated pressures cause fluid absorption, pressures >200 mm Hg did not result in fluid absorption, yet a rapid procedure under normal pressure conditions did.

We will continue to collect data on more patients, in collaboration with other hysteroscopists, to further determine which procedures are at risk and which are free of risk, of fluid absorption. We will also see if there is a pressure threshold above which fluid absorption occurs, keeping in mind the last patient cited, where symptomatic fluid overload occurred well within safe pressures.

Our conclusions thus far are as follows: (1) Significant intravasation of fluid may occur with extensive resections which open uterine venous channels; (2) significant intravasation also can occur at low pressures in the presence of unrecognized perforations; (3) alcohol is a reliable indicator for measuring fluid absorption and may be used to monitor this risk; (4) there appears to be a minimal risk of absorption during hysteroscopy under normal operative conditions.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

Dr. Ronald L. Brown, Charlotte, North Carolina. With increasing use of operative hysteroscopy as a surgical alternative for various gynecologic problems, there has become a heightened awareness of the potential complications associated with the procedure. The use of lasers and electocoagulation devices within the intrauterine cavity certainly poses a potential threat to the integrity of the uterine walls, which if violated can lead to a life-threatening situation. Fluid overload, as a result of excessive absorption of distention medium, is a potentially serious complication of hysteroscopy, the cause of which has never been fully understood. Goldrath was a pioneer of endometrial ablation with the Nd:YAG laser, and he recognized fluid overload in several of his patients. Goldrath et al.[1] hypothesized that insufficient dilatation of the cervix and prolonged operating time may be responsible for excessive fluid absorption. Others have suggested that if the fallopian tubes are not blocked that fluid will pass through them and into the peritoneal cavity and be absorbed into the circulation.

In 1990 Garry[2] reported a study of 63 women undergoing endometrial ablation. In 30 of these women fluid was infused with a simple roller pump, and in 33 women the pump was connected to a pressure transducer that maintained intrauterine pressure at a preset level or less. The pressure-controlled group showed an 86% reduction in the volume absorbed, which was measured by inflow-outflow volumes. He suggested that damaged peripheral uterine arteries were the pathway for fluid absorption and if intrauterine pressure was maintained at the patient's mean blood pressure or less, there would be no retrograde flow into the systemic circulation and no fluid overload.

Dr. Vulgaropulos has reported a clinical study of 12 patients in a well-designed protocol to directly measure fluid absorption and intrauterine pressure. This clinical study has been complemented by an experimental laboratory model that demonstrates the effects of several variables on intrauterine pressure and flow rates. It was shown very clearly in the experimental model that intrauterine pressure is primarily a function of the outflow resistance regardless of pump setting. If outflow

was unobstructed, intrauterine pressure was never >60 mm Hg. Conversely, closure of the outflow valve and cervix resulted in pressures >117 mm Hg at all pump settings.

In the clinical study 11 patients underwent various types of operative hysteroscopy and were maintained at intrauterine pressures of 45 to 75 mm Hg with ethanol-labeled glycine as the distention medium. At an undesignated time during the surgery and for an unspecified length of time, outflow of glycine was obstructed in some of these patients, generating intrauterine pressure of 230 and 280 mm Hg at different pump settings. In none of the 11 patients was there any evidence of fluid absorption. In a twelfth patient there was evidence of fluid absorption as measured by blood alcohol level. It is unfortunate that no intrauterine pressures were measured on this patient, but it is safe to assume that normal operating pressures (i.e., pressures >75 mm Hg) were exceeded and probably >200 mm Hg because outflow was totally obstructed. In this patient there was apparent excessive intrauterine pressure for 13 minutes at a time in the surgery when there were obviously open, damaged uterine vessels, and she did have evidence of fluid absorption. In the other 11 patients in the study there is no mention of how many had excessive intrauterine pressure and for how long the elevated pressures were present. It may also be of value to know at what point during the surgery excessive pressures were exerted (i.e., was there excessive intrauterine pressure after the resection of the tissue?). I would ask that Dr. Vulgaropulos comment on this and give his opinion of the significance of these temporal relationships.

It would seem that the cause of fluid absorption during hysteroscopic surgery is multifactorial. Certainly, perforation of the fundus of the uterus and cervical tears have been shown in other case reports to lead to fluid absorption and overload, as was the case with the two nonstudy patients in this report. The cardiovascular status, age, and hormone effect of the patient may also be factors.

Dr. Vulgaropulos's study has answered a number of questions about pressures, flow rates, and pumps used in operative hysteroscopy; however, I don't think we can as yet rule out excessive intrauterine pressure as having at least some role in fluid absorption in some patients.

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DR. VULGAROPULOS (Closing). The temporal relationship and the timing is something that we have not quite worked out in our study design. The time the procedure takes is much the same except for the quicker diagnostic procedures. Regarding the elevated pressures, we are just trying to get an idea of how much pressure can be created, and we are holding them for 30 to 60 seconds as opposed to doing the complete resection with closed outflow. After the resection is done, we are basically still at open outflows but it is just within the procedure itself that we will close down the outflow to measure and see what pressures we get for a period of time. It appears that the patient with fluid absorption had it occur over a 10-minute period.

Obesity and lower urinary tract function in women: Effect of surgically induced weight loss

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OBJECTIVE: The subjective and objective effects of massive weight loss on lower urinary tract function in morbidly obese women were examined.

STUDY DESIGN: Thirteen subjects underwent a comprehensive evaluation of lower urinary tract function before and 1 year after surgically induced weight loss.

RESULTS: We demonstrated significant improvements in lower urinary tract function after weight loss. Of 12 subjects who complained of incontinence before surgery only three complained of incontinence ($p = 0.004$) and only one requested treatment after weight loss. Objective and subjective resolution of both stress and urge incontinence was documented. Statistically significant changes were seen in measures of vesical pressure, the magnitude of bladder pressure increases with coughing, bladder-to-urethra pressure transmission with cough, urethral axial mobility, number of incontinence episodes, and the need to use absorptive pads.

CONCLUSION: Weight reduction is desirable for obese women complaining of urinary incontinence and may obviate the need for further incontinence therapy. (Am J Obstet Gynecol 1992;167:392-9.)

Key words: Urinary incontinence, stress incontinence, detrusor instability, obesity, weight loss

Obesity is commonly cited as a factor related to the development and recurrence of urinary incontinence in women.[1-3] There are relatively few studies that have objectively examined this relationship, however. In 1988 Dwyer et al.[4] compared the prevalence of obesity in 368 incontinent women (232 with genuine stress incontinence and 136 with detrusor instability) with 1981 population norms for Australia. They found that obesity, defined as >120% of the average weight for height and age, was significantly more prevalent in both women with genuine stress incontinence and those with detrusor instability than in the normal population. They were unable to show any differences between obese and nonobese incontinent subjects on urodynamic testing, which included cystometry and uroflowmetry. In the same year Kolbl and Riss[5] examined the relationship between several indexes of body mass and genuine stress incontinence in 150 stress incontinent women and 43 continent controls. They found no significant differences in any of the indexes between the continent and incontinent groups. During urodynamic testing they demonstrated that subjects with a positive stress test had a significantly higher body mass index than subjects with a negative stress test and that an increased body mass index was associated with a significantly greater resting total urethral pressure but not with a greater resting or stressed urethral closure pressure. In a paper presented before the American Uro-Gynecologic Society in 1987 Wilkie and Stanton[6] reported the results of passive and dynamic urethral pressure profiles in five obese continent, five nonobese continent, five obese stress incontinent, and five nonobese stress incontinent women. Although they found significant differences in urethral measurements between the stress continent and incontinent groups, no significant differences were demonstrated between the obese and nonobese subgroups. Finally in 1988 Deitel et al.[7] reported a significant decrease in the prevalence of the symptom of stress incontinence (from 61.2% to 11.6%) in 138 morbidly obese women after weight reduction induced by bariatric surgery. No objective testing was performed.

In summary, previous studies have demonstrated disparate relationships between obesity and objectively documented incontinence. None have shown significant differences due to obesity in various urodynamic measurements. The only study to examine the relationship between successful weight loss and incontinence was entirely subjective.

The aims of the present study were to examine the subjective and objective effects of massive surgically

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induced weight loss on lower urinary tract function in morbidly obese women.

Material and methods

Eighteen morbidly obese women who were considered candidates for bariatric surgery volunteered to participate in this study, which was approved by the Medical College of Virginia Committee on the Conduct of Human Research. To be a candidate for surgery, a subject had to be at least 45 kg above her ideal body weight as estimated by the 1983 Metropolitan Life Insurance tables for medium frame. Each subject completed approved consent procedures before initial evaluation. All underwent a comprehensive evaluation of lower urinary tract function as detailed below. After extensive medical, nutritional, and psychologic evaluation and counseling, 13 of the subjects underwent gastric bypass surgery; these women comprise the final study population.

The evaluation of lower urinary tract function included the completion of a detailed urogynecologic history, the performance of a genitourinary physical and neurologic examination, the completion of a prospective 1-week urinary diary,[8] and the performance of a standardized fluid loss quantitation test[9] and urodynamic evaluation. The urodynamic evaluation included uroflowmetry with postvoid residual urine volume measurement, retrograde provocative multichannel water urethrocystometry (in 45 degree upright, sitting, and standing positions) with microtip transducer catheters, passive (at rest) and dynamic (while coughing) urethral pressure profilometry, resting and stressed urethral axis determinations, and direct visualization testing for fluid loss. Specific details of the urodynamic methods have been published previously.[10-12] This entire evaluation was performed before gastric bypass surgery and repeated once weight loss had stabilized after surgery. The repeat evaluations were performed a mean of 14.5 months (SD 3.6, range 11 to 24) after the gastric bypass. No subject received any medical, surgical, or behavioral therapy for incontinence during the time between initial and repeat evaluations.

A subject was considered to have the symptom of stress incontinence if she complained of the objectionable and involuntary loss of urine coincidental with physical activity. The diagnosis of the condition of genuine stress incontinence was made if the subject had the symptom of stress incontinence and had observable leakage produced by stress without concurrently demonstrable detrusor activity during urethrocystometry or had a positive direct visualization test immediately after the catheters were removed in the total absence of detrusor instability during urethrocystometry. A subject was considered to have the symptom of urge incontinence if she complained of the involuntary loss of urine associated with the strong desire to urinate. The diagnosis of the condition of detrusor instability was made if the subject had the symptom of urge incontinence and had the objective demonstration of a detrusor contraction and urinary leakage, spontaneously or on provocation, during urethrocystometry while she was attempting to inhibit micturition. A subject was considered to have the symptom of mixed incontinence if she had the symptoms of both stress incontinence and urge incontinence. The diagnosis of the condition of mixed incontinence was made if the diagnostic criteria were fulfilled for both genuine stress incontinence and detrusor instability. Urodynamic techniques and measurements, terms, and diagnostic criteria conform to the recommendations of the International Continence Society.[13]

Quantitative data were analyzed for significance with the two-tailed paired t test. Unless otherwise noted, all quantitative data are expressed as the mean ± SEM. Simple regression analysis was used to analyze the relationship between body mass index and various measures of vesical pressure. Categorical data were analyzed for significance either with McNemar's χ^2 test or the two-tailed Fisher's exact test as dictated by expected cell size.

Results

The thirteen subjects who completed the study had a mean age of 41 years (SD 11.9, range 22 to 65); 10 were premenopausal and three were postmenopausal. The mean parity was 2.2 (SD 1.3, range 0 to 4) and the mean height was 1.63 m (SD 0.06, range 1.52 to 1.73). The mean preoperative weight was 131.5 kg (SD 19.9, range 99.8 to 153.3). The mean preoperative body mass index (calculated by dividing the weight in kilograms by the square of the height in meters) was 49.4 kg/m^2 (SD 7.9, range 38.6 to 62.2). All subjects experienced significant weight loss after gastric bypass, with a mean postoperative weight of 88.1 kg (SD 17.0, range 59.9 to 114.8, $p = 0.0000009$) and a mean postoperative body mass index of 33.1 kg/m^2 (SD 6.7, range 21.2 to 38.6, $p = 0.000001$).

Table I lists the preoperative and postoperative symptoms and urodynamic diagnoses of each of the 13 subjects. Twelve subjects (92%) had bothersome symptoms of urinary incontinence before surgery. After surgery only three subjects (23%) complained of incontinence ($p = 0.004$). Seven of 10 subjects with the symptom of stress incontinence ($p = 0.02$) and eight of nine subjects with the symptom of urge incontinence ($p = 0.004$) noted resolution of the symptom. The three subjects with persistent symptoms all had mixed incontinence before surgery; two noted resolution of the urgency incontinence component of their symptoms after weight loss.

Table I. Summary of subjects' symptoms and conditions before and 14.5 (SD 3.6, range 11 to 24) months after gastric bypass surgery

	Before surgery	*After surgery*	*No. subjects*
Symptoms	No incontinence	No incontinence	1
	Urge incontinence	No incontinence	2
	Stress incontinence	No incontinence	3
	Mixed incontinence	No incontinence	4
	Mixed incontinence	Stress incontinence	2
	Mixed incontinence	Mixed incontinence	1
Conditions*	Normal	Normal	7
	Detrusor instability	Detrusor instability	1
	Genuine stress incontinence	Genuine stress incontinence	1
	Genuine stress incontinence	Normal	2
	Detrusor instability	Normal	1
	Genuine stress incontinence + detrusor instability	Normal	1

*Urodynamic diagnosis.

Only six of the 12 subjects with symptoms of incontinence before weight loss had objective documentation of incontinence during urodynamic testing (Table I). Four of the six had resolution of urodynamic abnormality after weight loss, three of four subjects with genuine stress incontinence and two of three subjects with detrusor instability. None of these differences in presurgical and postsurgical urodynamic diagnoses achieved statistical significance.

Of the twelve subjects with symptoms of objectional incontinence before weight reduction, only one desired treatment at the time of repeat evaluation. This was a 22-year-old woman with lifelong symptoms of mixed incontinence and enuresis. Her weight decreased from 160 to 107 kg, but she had persistent symptoms and persistent detrusor instability on urodynamic testing. She responded well to imipramine and a program of bladder retraining. Neither of the two subjects with persistent though diminished symptoms of stress incontinence, one of whom had persistent genuine stress incontinence on testing, desired treatment. They had both noted a decrease in the number of incontinence episodes per week, one from 18 to two and the other from four to one.

On the 1-week prospective urinary diary (Fig. 1), there were significant decreases in the number of incontinence episodes per week before and after weight loss (13.4 ± 2.9 vs 0.9 ± 0.5, $p = 0.001$) and in the number of incontinence pads used per day (2.0 ± 0.5 vs 0.08 ± 0.08, $p = 0.00008$). There were no significant differences in daytime voiding frequency (every 2.9 hours before surgery vs every 3.3 hours after surgery), or in episodes of nocturia per week (8.2 before surgery vs 6.9 after surgery).

During urodynamic testing all intravesical pressure measurements before weight loss were significantly greater than those after weight loss (Fig. 2). These included (expressed in centimeters water pressure) the initial pressure in the 45 degree upright position (40.3 ± 3.7 vs 25.3 ± 1.7, $p = 0.0007$), pressure at maximum cystometric capacity in the same position (45.0 ± 3.1 vs 27.2 ± 1.9, $p = 0.00006$), pressure sitting upright (56.1 ± 2.9 vs 32.9 ± 2.1, $p = 0.000006$), pressure standing (56.7 ± 3.4 vs 32.8 ± 2.1, $p = 0.00001$), and the increase in pressure from 45 degrees to standing (13.2 ± 1.4 vs 6.8 ± 1.0, $p = 0.005$). These changes were due to decreases in abdominal pressure rather than to decreases in true detrusor pressure. There was a significant positive correlation between body mass index and initial vesical pressure ($r = 0.50$, $p = 0.01$), pressure at maximum cystometric capacity ($r = 0.57$, $p = 0.003$), sitting pressure ($r = 0.60$, $p = 0.001$), and standing pressure ($r = 0.60$, $p = 0.001$).

There were also significant differences seen in all cough-related parameters before and after weight loss (Fig. 3). The increases in vesical pressure with four maximum effort coughs were significantly greater before weight loss (greatest amplitude cough 104 ± 10 cm water, mean of four coughs 93 ± 10) than after surgery (greatest 76 ± 6, mean of four 68 ± 5, $p = 0.001$ and 0.004, respectively). Cough pressure transmission from the bladder to the urethra was significantly improved (higher) after weight reduction (83% ± 3% before surgery vs 93% ± 5% after surgery, $p = 0.03$). Finally, urethral axial mobility with coughing was significantly less after weight reduction (stressed cotton swab angle 37 ± 4 degrees before vs 24 ± 4 degrees after surgery, $p = 0.005$).

There were no significant changes related to weight loss in any other urodynamic parameter, including measures of passive urethral function (functional urethral length or maximum urethral closure pressure), bladder capacity (first sensation of fullness and maximum cystometric capacity), and emptying function (mean and maximum flow rates and postvoid residual volumes).

No significant differences were noted during quantitation testing; however, the power to detect a difference was very low. Six subjects lost no fluid either before

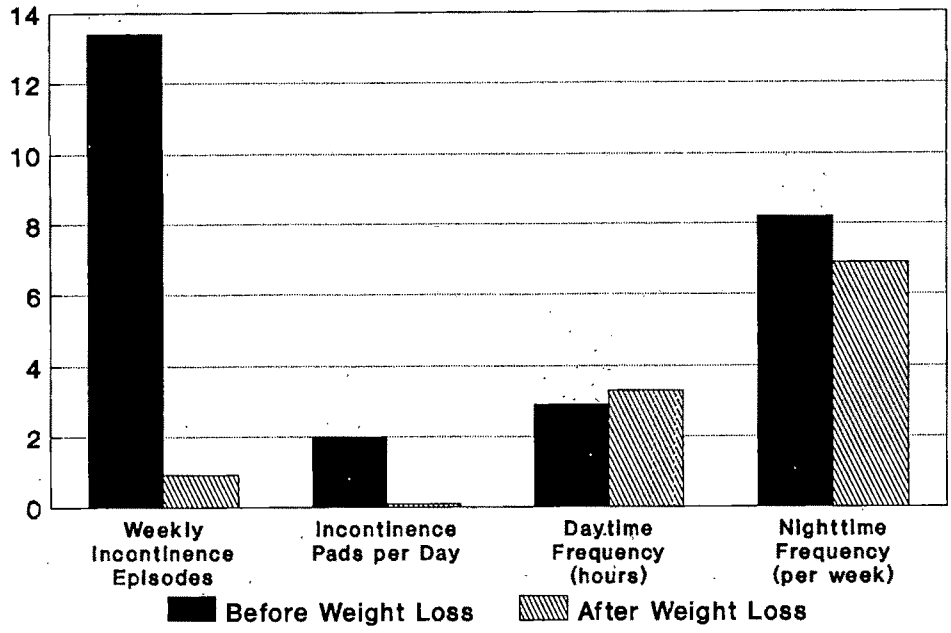


Fig. 1. Effects of surgically induced weight loss on number of incontinence episodes per week ($p = 0.001$), number of incontinence pads used per day ($p = 0.00008$), daytime voiding frequency in hours, and episodes of nocturia per week.

or after weight reduction and five subjects who lost 2, 5, 6, 35, and 173 gm before weight reduction recorded no loss after. One subject with persistent genuine stress incontinence lost 162 gm before and 147 gm after surgery; another with persistent detrusor instability lost 81 gm before and 226 gm after.

Comment

This study has demonstrated both statistically and clinically significant improvements in lower urinary tract function after marked, surgically induced weight reduction in morbidly obese women. This improvement has been seen in both subjective and objective parameters. Subjectively, nine of 12 women with bothersome incontinence before weight loss noted complete resolution of their incontinence after weight loss. Moreover, two of the three subjects with persistent symptoms of incontinence had noted improvement to the point that they desired no treatment for their residual incontinence. Only one of 12 incontinent subjects required treatment after weight reduction; she had had lifelong mixed incontinence and enuresis. Objectively, two of three subjects had resolution of pure genuine stress incontinence, one of two had resolution of pure detrusor instability, and one had resolution of mixed genuine stress incontinence plus detrusor instability on urodynamic testing. Although these changes did not reach statistical significance due to the small number of subjects, they had obvious clinical significance for the four women who normalized their studies. Our findings are in agreement with prior studies by Dwyer et al.,[4] which suggested that obesity contributed both to genuine stress incontinence and detrusor instability, and by Deitel et al.,[7] which showed that massive weight loss often led to the resolution of the symptom of stress incontinence.

Urodynamically we have demonstrated that massive weight loss in morbidly obese women significantly reduced intravesical pressure in all positions and reduced pressure increases seen with standing. Moreover, pressure increases with coughing were also significantly less following weight loss. Finally, there were statistically significant improvements in the stress competence (reflected by an increase in the pressure transmission ratio) and in the anatomic stability (reflected by a decrease in stressed Q-tip cotton swabs angle) of the urethra after weight loss. These are all measurements that were not recorded in previous urodynamic evaluations by Dwyer et al.[4] or by Kolbl and Riss.[5] In agreement with these authors, we did not see significant differences in other cystometric, urethral, or uroflowmetric measurements. Our results do differ from those of Wilkie and Stanton,[6] who demonstrated no differences in bladder pressure with position change or cough and no differences in passive or dynamic urethral function when comparing obese and normal-weight subjects. This discrepancy is likely explained by differences in study design, in that our comparison of the same subjects before and after weight loss minimizes many confounding variables that might have influenced these measures in Wilkie and Stanton's small study groups.

Our urodynamic results do suggest that the mechanism for improved stress continence in our subjects is related to lesser increases in abdominal pressure with

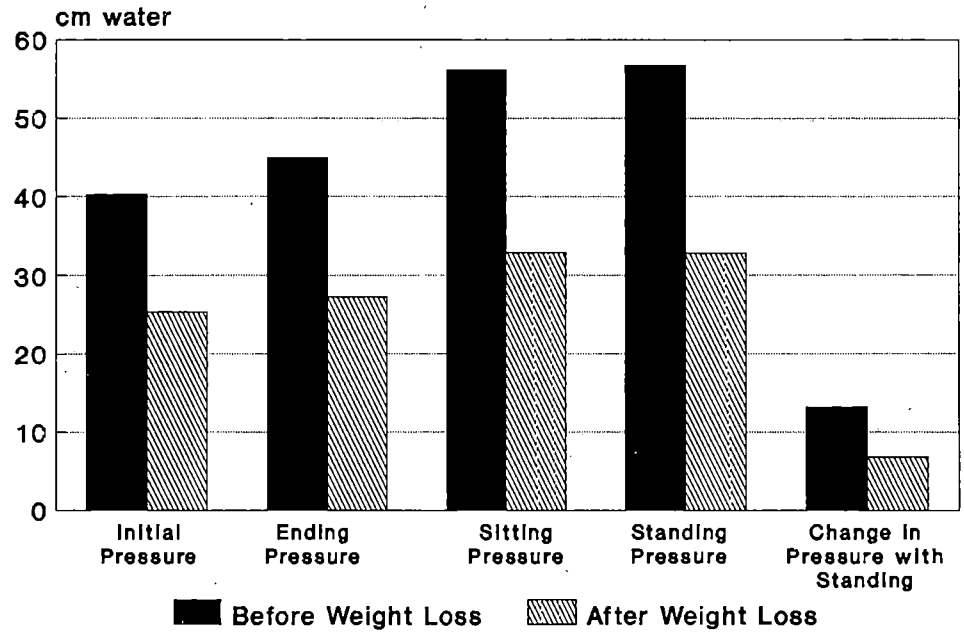


Fig. 2. Effects of surgically induced weight loss on intravesical pressure measurements at beginning of cystometry ($p = 0.0007$), at maximum cystometric capacity ($p = 0.000006$), sitting ($p = 0.000006$) and standing ($p = 0.00001$) at maximum cystometric capacity, and on the increase in pressure with standing ($p = 0.005$).

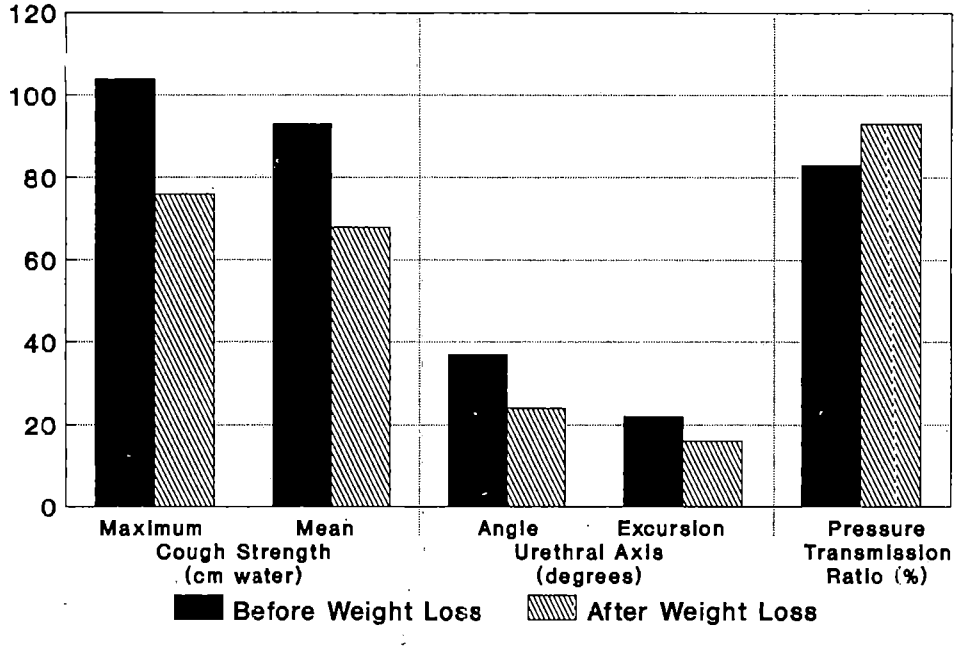


Fig. 3. Effects of surgically induced weight loss on maximum ($p = 0.001$) and mean (0.004) intravesical pressure changes, urethral axis ($p = 0.005$) and excursion arc ($p = 0.04$) and bladder to urethra pressure transmission ratios ($p = 0.03$) with coughing.

physical stress, improved transmission of this stress to the urethra, and decreased axial mobility of the urethra. All of these factors are felt to be important in the pathophysiology of genuine stress incontinence.[11] The data provide no indication as to the mechanism for the resolution of urge incontinence and detrusor instability. We are aware of no evidence that decreases in total bladder pressure, such as observed in this study, will improve one's ability to suppress detrusor instability. It is possible that alterations in autonomic nervous system activity related to obesity described in animals and humans[14] may normalize with weight reduction. Increased sympathetic and decreased parasympathetic stimulation to the lower urinary tract could consequently promote enhanced detrusor stability.

Two qualifying aspects of this study must be men-

tioned. The first is the profound degree of obesity demonstrated by our population. Obesity is defined formally by a body mass index >30 kg/m^2.[15] Our population's mean body mass index of 33.1 after weight reduction reflects the severity of its obesity; only five of 13 subjects achieved a body mass index of ≤30 after weight loss. We cannot be certain that our findings and conclusions can be generalized to the 10% to 12% of Americans who are defined as obese,[15] most of whom are significantly less obese that our study population.

The second qualification is our inability to establish an objective diagnosis of incontinence in half of the 12 subjects who complained of the symptom of incontinence. This compares with a nondiagnostic study rate of 9.5% (18/188) for other patients complaining of incontinence studied by the first author during the same time with the same diagnostic criteria and to a 10.3% (15/145) rate reported in a previous study from our laboratory.[16] This discrepancy may reflect technical difficulties in establishing the diagnosis of incontinence, particularly in directly observing leakage, in massively obese subjects. However, it may also reflect confusion regarding the source of perineal wetness in such women. In any event, we feel that this observation should generate a degree of skepticism regarding conclusions based on studies that use only subjective measures to study incontinence in obese subjects.

With the findings and qualifications of this study in mind, we conclude that weight reduction is desirable for obese women complaining of urinary incontinence. Successful weight reduction may obviate the need for further incontinence therapy in at least some obese patients. In addition, for obese women with genuine stress incontinence who undergo continence surgery, weight reduction may improve the technical ease, increase the durability, and decrease the failure rate of such surgery.[4] A comprehensive survey of the treatment of obesity and the role of surgery in the treatment of severe obesity is beyond the scope of this paper; the interested reader is referred to a recent National Institutes of Health consensus statement on this topic.[17] However, it must be emphasized that traditional diet and exercise treatment of obesity is often unsuccessful, with permanent cure realized by fewer than 10% of patients after a decade.[15] Thus for some morbidly obese incontinent women gastric bypass surgery would seem preferable to and of greater overall benefit than continence surgery.

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Discussion

DR. JOHN W. LANE, Chapel Hill, North Carolina. In spite of the fact that we gynecologists are taught, beginning in medical school, that obesity contributes to female urinary incontinence, objective documentation of this is surprisingly lacking on our literature.[1, 2] Nor is there any prior objective prospective study which supports the recommendation of weight loss to the obese patient to reduce her incontinence symptoms. This is the first study that approaches this in a systematic, objective, prospective manner.

Bump et al. have reported a very interesting study of a group of extraordinary patients. They were enormously obese women, having an unusual procedure with which most gynecologists have little if any experience. Nevertheless, the experience with these patients can help us to understand the relationships between

obesity, weight loss, and female urinary incontinence. This study may have use for us in caring for the much more extensive group of obese women who are not as large, but with whom we are all well acquainted.

Most gynecologists would advise weight loss to obese patients as one part of a conservative program to help diminish or eliminate symptoms of incontinence, but objective evidence of improvement in urodynamic function after weight loss has heretofore been scanty to absent. Such evidence as does exist is fairly, clearly, and concisely reviewed by Dr. Bump in the introduction.

The purpose of this study, clearly stated, was to evaluate both subjective and objective effects of significant weight reduction the lower urinary tract function of obese women.

The patient selection was direct. Thirteen patients who were selected for gastric bypass surgery for weight loss entered the study. We are not told how many other patients were invited to participate but declined. Although a wide range of weights were included (99.8 to 153.3 kg) all were markedly obese as demonstrated by the mean weight of 131.5 kg and the fact that each was a minimum of 45 kg above ideal weight for medium frame.

This is an unique group of obese patients because each of them could reliably be expected to lose a large amount of weight because of surgery, thus making it possible to focus the study on subjects who would have successful weight loss. We can imagine how many subjects would have had to be studied to find a group this large that would successfully attain this degree of weight loss with traditional methods of diet and exercise.

The definitions and explanations of the terms used and the diagnostic criteria are succinctly stated and clearly explained. All techniques, parameters, measurements and criteria conformed to internationally established standards.

The patients underwent thorough and extensive lower urinary tract evaluation. The urodynamic studies are described in understandable and exacting detail. All studies were performed before surgery and repeated after the weight loss had stabilized after surgery. No other therapies were undertaken in the interim.

Of the 13 subjects, 12 reported preoperative incontinence. Nine of these 12 no longer complained of bothersome incontinence when reevaluated 1 to 2 years postoperatively. Three were still incontinent, although two of these were improved.

Of interest is the fact that six of the 12 subjects with symptoms of incontinence had normal urodynamic studies both before and after weight loss. The remaining six patients did have objective documentation of incontinence during testing. Of these six, four had resolution of their urodynamic abnormality.

There were three patients who still complained of incontinence, two of whom had objective incontinence and one with symptoms of incontinence. Two were improved and only one (with objective detrusor instability) sought further treatment.

Although statistical significance was not achieved due to the small numbers, one must assume that the patients who improved were pleased with this result. Even the six patients with symptomatic incontinence alone must have been delighted with remaining dry and were probably not at all concerned that their improvement was not confirmed by objective measurements.

The urodynamic studies support the view that the improvement was due to decreased intraabdominal pressure, increased pressure transmission to the urethra, and decreased urethral mobility. The authors submit that the improvement in detrusor instability may have been due to normalization of the effects of the autonomic nervous system on the lower urinary tract.

The authors warn us regarding two aspects of the study. First, these patients were massively obese. We cannot be certain that the same results would occur in more "normally" obese patients, which, of course, make the bulk of the obese population. Second, half of the subjects who complained of incontinence could not be shown objectively to have it. The nondiagnostic study rate is usually between 10% to 30%.[3] Several possible reasons for this were reviewed.

We can certainly agree that obese women with urinary incontinence should be counseled to lose weight as part of a conservative program to improve their symptoms. They may, indeed, need no further therapy. However, even if incontinence should persist after weight loss, surgery should then be technically less difficult, less risky, and more likely to be a long-term success.

I would like to ask Dr. Bump if there were many other gastric bypass patients who were invited to participate in this study but who declined.

I would also be interested to know if you have continuing studies on additional gastric bypass patients, or perhaps studies on less obese patients in more traditional weight reduction programs.

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Dr. A. Cullen Richardson, Atlanta, Georgia. You didn't specify how many of these women were parous.

Dr. Bump (Closing). A number of patients were offered the opportunity for free urodynamic testing. The ones who refused were all continent. We had hoped initially to look at a group of incontinent and a group of continent women, but as you see only one of the 13 was continent. We would have liked to have compared test results after weight loss in those who did not complain of incontinence.

The study is not continuing. The most practical reason is that the motor on our urodynamic chair was in danger of burning out. It usually took me, the nurse,

and the resident to tip the chair backward, assisting the grinding gears. We did a gastric bypass study because our chances of finding a group of 13 women who are going to lose over one half ton of weight in 14 months is relatively low; Dr. Sugerman is pretty good at achieving that weight loss for us.

One of the five patients who was initially evaluated but decided not to go ahead with bypass surgery is actually continent now, having lost a little over 52 kg on her own through a weight loss and exercise program. Another three who did not change their weight report that they had no change in their symptoms. One could not be located.

Dr. Richardson, one subject was nulliparous. The mean parity was 2.2. Interestingly, all of these patients had delivered vaginally. There were no cesarean sections in any of the subjects.

The incidence of genital prolapse after the Burch colposuspension

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OBJECTIVE: Our objective was to determine the incidence of postoperative genital prolapse after the Burch colposuspension and to identify risk factors for the development of subsequent prolapse.
STUDY DESIGN: The charts of 131 patients who had a Burch colposuspension performed by the senior author (S.L.S.) between 1977 and 1986 were reviewed at the Urodynamic Unit of St. George's Hospital, London. Emphasis was placed on the degree of genital prolapse on clinical examination and whether further surgery was required to correct the prolapse.
RESULTS: Thirty-five patients (26.7%) required a total of 40 operations to correct genital prolapse after colposuspension. At 20 operations, more than one procedure was required to correct combined prolapse. The patient's age, weight, parity, menopausal status, and prior pelvic surgery did not affect the incidence of postoperative prolapse. The only preoperative risk factor identified was the presence of a large cystocele.
CONCLUSION: Postoperative genital prolapse is a significant complication of the Burch colposuspension. It is unclear whether this is due to a disruption of the vaginal axis or to an intrinsic weakness of the pelvic floor in these women. (Am J Obstet Gynecol 1992;167:399-405.)

Key words: Stress incontinence, prolapse, Burch colposuspension

Although there are many operations used for the treatment of urethral sphincter incompetence (genuine stress incontinence), the Burch colposuspension is one of the most popular and most successful. The first retropubic urethrovesical suspension was described by Williams in 1947, who placed sutures between the bladder wall and the periosteum of the symphysis.[1] In 1949 Marshall et al.[2] described their operation in which sutures are placed between the vaginal wall and periosteum and cartilage of the symphysis. Hampered by sutures pulling out of the periosteum with this technique, Burch,[3] in a procedure published in 1961, suspended the paravaginal fascia first to "the white line" of the levator fascia and eventually to Cooper's ligament. Subsequently, the Burch operation has been called by many different titles, although it appears the term colposuspension was first used by Warwick and Whiteside in 1970.[4]

In spite of the success achieved with the Burch colposuspension, with incontinence cure rates ranging from 71% to over 95% reported,[4-17] there are several recognized complications resulting from the operation. These include postoperative detrusor instability and

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voiding disorders.[4, 7, 12, 18-21] One of the less noted complications is the occurrence of enterocele and rectocele. In Burch's original description of the procedure, he states that in cases of doubt it is best to repair the posterior vaginal wall at time of colposuspension. After the operation he noticed an elevation in the posterior vaginal wall just in front of the cervix that appears after the anterior vaginal wall is elevated. He thought this change predisposed to enterocele formation.[3] This tenting of the posterior vaginal wall after colposuspension has also been described by Stanton and others as a cause of dyspareunia and as an aggravation of posterior vaginal wall weakness.[4, 11, 12, 14, 16]

The incidence of enterocele after the Burch procedure reported in the literature ranges from 3% to 17%, but there are little data on postoperative rectocele development and uterine descent.[1, 3-6, 11-17] Furthermore, there is sparse information on factors that may predispose patients to genital prolapse after a Burch operation and little consensus on the appropriate operative management of this potentially significant complication. With these issues in mind, we reviewed the outcome of patients who had a colposuspension operation, specifically focusing on the issue of postoperative prolapse.

Material and methods

The medical charts of 131 consecutive patients who had a Burch colposuspension operation for the treatment of genuine stress incontinence between 1977 and 1986 were reviewed at the Urodynamic Unit of St. George's Hospital, London. All of the operations were done or supervised by the senior author (S.L.S.) at either St. James Hospital, St Helier Hospital, or St. George's Hospital. Information sought from the charts included the age, weight, parity, menopausal status, and estrogen therapy (if applicable) of the women before surgery. Previous incontinence surgery, prolapse surgery, or hysterectomy were noted. All patients had preoperative urodynamic studies that included a cystometrogram or voiding cystourethrogram and uroflowmetry. The degree of genital prolapse was carefully evaluated by S.L.S. preoperatively and corrected at the time of the colposuspension, if significant. Postoperatively, the return of satisfactory bladder function as determined by the removal of the suprapubic catheter, the return of bowel function, the total number of hospital days, and any special complications were recorded. After discharge, the patients were usually seen at 2 to 3 months after surgery for clinical review and repeat urodynamic studies. Thereafter, they were seen annually for 5 years for review of symptoms, pelvic examination, uroflowmetry, and a pad test. At postoperative follow-up review in this study, the emphasis was on the degree of genital prolapse on clinical examination and whether further surgery was required to correct the prolapse. Patients who had not been examined in the past year were asked to come to the urodynamic unit at St. George's Hospital for evaluation. Only those patients with complete medical notes, a recent examination, and a follow-up period of >3 years were included in the study.

The patients were divided into two groups. Group 1 included the patients who did not require additional surgery for genital prolapse after the colposuspension operation. The patients who did have corrective surgery for prolapse were placed in group 2. The two groups were compared for any factor that may predict genital prolapse after colposuspension, with the hope of identifying preoperatively patients who may be at increased risk.

A modified Burch colposuspension, as previously described by Stanton,[4-6] was performed in all patients. After the induction of anesthesia, the patient was placed in the horizontal lithotomy position with the legs supported by Lloyd-Davis stirrups. Cystoscopy was performed initially in all patients to ensure they had no condition that would complicate surgery. If cystoscopy was satisfactory, the abdomen and perineum were prepped and draped in a sterile fashion. A transuretheral resection drape was used to cover the perineum, allowing access to the vagina with the sterile condom during the operation. A 14F Foley catheter was placed in the bladder to allow continuous drainage during the operation and to facilitate the delineation of the urethra and the bladder neck.

Access to the retropublic space was obtained through a low Pfannenstiel or Cherney incision. If a hysterectomy, tubal ligation, or culdoplasty was needed, it was accomplished before the colposuspension, and the peritoneum was closed with a 2-0 continuous delayed absorbable suture. Visualization of the space of Retzius was enhanced by the use of a Denis Browne self-retaining ring retractor. The bladder was carefully dissected from the symphysis. With a forefinger in the vagina, the operator elevated the lateral vaginal fornix and dissected the bladder base medially off the paravaginal fascia. Although this can sometimes be accomplished bluntly, scissor dissection was often required. Three of four pairs of No. 1 nonabsorbable polybutylate-coated polyester sutures (Ethibond, Ethicon, Ltd., Edinburgh) were then placed into the paravaginal fascia on each side and inserted into the ipsilateral ileopectineal ligament. The most distal (caudad) suture was inserted opposite the bladder neck. The remaining sutures were placed cephalad along the bladder base about 1 cm apart. Once all the sutures were in place, they were tied, approximating the paravaginal tissue to Cooper's ligament. The most caudad sutures were tied first, and then the remainder of the sutures were tied on alternating sides to ensure balanced elevation.

Once adequate hemostasis was achieved, the incision

Table I. Surgical procedures used to correct uterovaginal prolapse after colposuspension

Procedure	*No. of patients*
Posterior repair	11
Transvaginal hysterectomy, posterior repair	6
Vaginal enterocele, posterior repair	5
Transabdominal hysterectomy	3
Transvaginal hysterectomy	3
Abdominal sacrocolpopexy	2
Abdominal sacrocolpopexy, posterior repair	2
Transvaginal hysterectomy, enterocele repair, posterior repair	1
Transvaginal hysterectomy, enterocele repair	1
Sacrospinous ligament fixation	1
Posterior repair, colposuspension	1
Transabdominal hysterectomy, colposuspension	1
Transabdominal hysterectomy, colposuspension, posterior repair	1
Transabdominal hysterectomy, abdominal sacrocolpopexy, posterior repair	1
Zacharin procedure, posterior repair	1

was closed. A suction drain was left in the retropubic space. A suprapubic catheter was placed two fingerbreadths above the incision after the bladder had been filled through the Foley catheter. The catheter was then removed. Any necessary repair of the posterior vaginal wall was then accomplished.

Postoperatively, the retropubic suction drain was removed 24 to 36 hours after surgery. The suprapubic catheter was clamped on day 2 to allow the patient to attempt to void spontaneously. The catheter was removed once she voided amounts >200 ml, and the residual urine was <150 ml.

Results

The age of the 131 study patients ranged from 30 to 76 years with a mean of 47 ± 9.8 years. All of the patients had urodynamically proved urethral sphincter incompetence, although four patients also had a component of detrusor instability.

Group 1 consisted of 96 patients (73.3%) who did not require surgery for genital prolapse after colposuspension operation. Thirty-five patients (26.7%) required corrective prolapse surgery and were placed in group 2.

The surgical procedures used to correct postcolposuspension prolapse are listed in Table I. Of the 131 patients, 29 patients (22.1%) had a rectocele repair, 14 (10.7%) underwent an enterocele repair, 17 (13.0%) had a hysterectomy, and three (2.3%) had a repeat colposuspension subsequent to the initial colposuspension. A total of 40 operations were performed with 31 patients requiring one additional surgery, three patients had two additional surgeries, and one patient required three subsequent operations to correct genital prolapse. At 20 operations, more than one procedure was required to correct combined prolapse (i.e., enterocele and rectocele repair). The average time interval between the initial colposuspension and the operation to correct symptomatic prolapse was 40.23 ± 26.35 months (range 9 to 70 months). The period of follow-up in the study ranged from 3 to 14 years with a mean of 5.1 ± 1.41 years. Overall, 81.8% of the patients in the series were cured of stress urinary incontinence, 82.3% in group 1 and 80.0% in group 2.

Table II. Primary operation

Operation	*Group 1 (N = 96)*		*Group 2 (N = 35)*	
	No.	*%*	*No.*	*%*
Colposuspension only	77	80.2	27	77.1
Colposuspension + additional operation	19	19.8	8	22.9
Transabdominal hysterectomy	7	7.3	3	8.6
Uterine suspension	1	1.0	—	—
Posterior repair	10	10.4	4	11.4
Moschcowitz and posterior repair	1	1.0	—	—
Moschcowitz	—	—	1	2.9

$p = 0.889$ (χ^2).

A comparison of the clinical characteristics between those who had corrective prolapse surgery and those who did not demonstrates no significant difference in age, weight, parity, or menopausal status. There were only three patients in the study on hormone replacement therapy; two in group 1 and one in group 2, so this obviously had no relevance in the study.

The incidence of previous surgery also did not appear to be a significant factor for the subsequent development of prolapse. In group 1, 51 patients (53.1%) had no previous pelvic surgery compared with 20 patients (57.1%) in group 2. Of those that had previous surgery, 45 patients in group 1 had 59 prior pelvic operations, whereas 15 patients in group 2 had 30 previous gynecologic surgeries. Twenty-five patients (26.0%) in group 1 had a previous hysterectomy compared with 13 (37.1%) in group 2, again not significantly different ($p = 0.215$).

A summary of the principal surgical procedures in the study is seen in Table II. Overall, 104 patients (79.4%) had a colposuspension only, whereas the remaining patients had a posterior repair, abdominal hysterectomy, or culdoplasty in addition to the colposuspension. One patient had a uterine suspension. Although the number of patients who had additional procedures at the time of the primary colposuspension was small, they were evenly distributed between the two

Table III. Preoperative vaginal prolapse

Symptom	Group 1 (N = 96)		Group 2 (N = 35)		p*
	No.	%	No.	%	
Cystocele					
Mild	57	59.4	18	51.4	
Marked	21	21.9	15	42.9	0.027
Rectocele					
Mild	53	55.2	16	45.7	
Marked	13	13.5	6	17.1	0.625
Enterocele					
Mild	16	16.7	6	17.1	0.949
Marked	—	—	—	—	

*χ^2, Pearson coefficient.

groups, and the additional surgery appeared to have no effect on the incidence of subsequent prolapse surgery. An analysis of the spontaneous voiding day, postoperative return of bowel function, the total number of hospital days, and the perioperative complications demonstrated no difference between groups 1 and 2.

Preoperatively, there was no difference between the two groups in the clinical symptoms associated with prolapse, such as a feeling of prolapse and the need for manual reduction of the posterior vaginal wall to facilitate bowel movements. Not surprisingly, the symptoms of prolapse were significantly greater in group 2 after the initial surgery ($p = 0.00001$), because these patients eventually required corrective prolapse surgery. The overall incidence of dyspareunia was decreased after colposuspension and was not significantly different between the two groups.

In the objective evaluation of uterovaginal prolapse in these patients, mild prolapse was defined as any descent of the vaginal wall or cervix with abdominal straining short of the vaginal introitus. Marked prolapse was defined as descent of the vaginal wall to the introitus or beyond. Preoperative assessment of vaginal prolapse revealed a significantly higher incidence of a marked cystocele in group 2 than group 1 ($p = 0.027$). There was no difference in the incidence of rectocele, enterocele, or uterine descent preoperatively (Tables III and IV). Because the percentage of patients with prolapse in each group exceeds 100% obviously there were some patients with combined prolapse (i.e., cystocele + rectocele or enterocele).

After colposuspension there was a significant decrease in the incidence of cystocele with only three patients (2.5%) found to have a marked cystocele, compared with 36 patients (27.5%) preoperatively (Table V). Just over one third of the patients, evenly distributed between groups 1 and 2, were found to have after colposuspension an asymptomatic, mild cystocele located near the uterine cervix or vaginal apex.

A significantly higher incidence of rectocele, enterocele, and uterine descent was found postoperatively in group 2 than in group 1, with 10 patients in group 2 developing a marked rectocele, 7 developing a marked enterocele, and 9 with marked uterine descent (Tables V and VI). As was noted preoperatively, many of the patients had combined prolapse. Of the 19 patients in group 2 who had an intact uterus at the time of their second operation (first operation to correct prolapse), 17 patients (89.5%) had the uterus removed as part of the procedure to correct their prolapse. It should be noted, however, that 64 patients (66.7%) from group 1 had an intact uterus that remained asymptomatic throughout the follow-up period after the colposuspension. Furthermore, there was no difference in the percentage of patients with an intact uterus preoperatively in groups 1 and 2, and the presence or absence of the uterus had no effect on the operation's incontinence cure rate.

Table IV. Preoperative uterine descent

Degree of uterine descent	Group 1 (N = 71)		Group 2 (N = 22)		p*
	No.	%	No.	%	
Mild	16	22.5	8	36.3	
Marked	1	1.4	—	—	0.450

*χ^2, Pearson coefficient.

Comment

The Burch colposuspension procedure is an effective surgical means to treat urethral sphincter incompetence and to correct anterior vaginal wall prolapse. Our series confirms the findings of previous studies that a cystocele is well corrected by a colposuspension.[3-5, 22] We were also able to confirm that uterine preservation does not adversely affect the ability of the operation to cure stress incontinence. The fact that there was no difference in the percentage of patients who had an intact uterus between the two groups suggests that uterine preservation does not predispose to postoperative prolapse. However, if a patient who develops prolapse has an intact uterus, the uterus is likely to require removal as part of the corrective prolapse surgery.

We were surprised to find that neither age, parity, weight, menopausal status, nor prior pelvic surgery predisposed to postoperative prolapse. Although the incidence of curing stress incontinence had been found to be lower in postmenopausal women than their premenopausal counterparts,[23] the incidence of postoperative prolapse appears no different in our series.

The only factor we were able to identify as a preoperative risk for the development of postoperative enterocele, rectocele, and uterine descent was the presence of marked anterior vaginal wall prolapse (i.e., a

Table V. Postoperative vaginal prolapse

Symptom	Group 1 (N = 96)* No.	%	Group 2 (N = 35)† No.	%	p‡
Cystocele					
Mild	35	36.5	10	28.6	
Marked	2	2.1	1	2.8	0.848
Rectocele					
Mild	51	53.7	14	40.0	
Marked	11	11.6	10	28.6	0.001
Enterocele					
Mild	20	21.1	10	28.6	
Marked	5	5.3	7	20.0	0.0002

*Last outpatient visit.
†Last outpatient visit before second operation.
‡χ^2, Pearson coefficient.

Table VI. Postoperative uterine descent

Degree of uterine descent	Group 1 (N = 64) No.	%	Group 2 (N = 19) No.	%	p*
Mild	17	26.5	3	15.8	
Marked	3	4.7	9	47.3	0.00000

*χ^2, Pearson coefficient.

cystocele that descends to the vaginal introitus or beyond), although the majority of patients had no identifiable risk factor. The elevation of the anterior vaginal wall that occurs with the Burch colposuspension may change the axis of the posterior vaginal wall, exposing it to a greater degree of intraabdominal pressure. A large cystocele would tend to be quite mobile with a resultant greater elevation of the anterior vaginal wall after colposuspension, which may result in a greater tendency for the disruption of the support to the vaginal apex and the posterior vaginal wall. These findings may also reflect an intrinsic weakness of the pelvic support tissues in these patients. Sayer et al.[24] performed surgical biopsies of the pubocervical fascia in 60 women with uncomplicated stress incontinence. They concluded that the collagen in stress incontinent women with bladder neck prolapse is weaker than those without prolapse. Because all of fascia of the pelvic floor is interrelated, it is reasonable to assume this weakness may extend to the fascia of the posterior vaginal wall and uterine supporting ligaments as well. If this is the case, there may be little that can be done to prevent the postoperative prolapse from developing. Although only 20.6% of the patients in our series had transabdominal hysterectomy, culdoplasty, or posterior repair at the time of colposuspension, the addition of these procedures did not decrease the incidence of subsequent corrective prolapse surgery. This was also the experience reported by Burch.[3] In his initial report of 53 cases, four patients (7.5%) developed postoperative enteroceles. Three of the four had a transabdominal hysterectomy and culdoplasty at the time of colposuspension.

Although this study seems to confirm the suspicion that the Burch colposuspension weakens the support to the vaginal vault and posterior vaginal wall, it is possible that other life events in these women may play a role. As the average time from colposuspension to corrective prolapse surgery was 3 years, the effects of entering menopause, weight gain, changes in life-style, and aging of tissues may have an influence on the development of prolapse; these factors are difficult to quantitate. However, the potential of this operation to predispose to genital prolapse through the alteration of the vaginal axis cannot be ignored.

In conclusion, postoperative enterocele, rectocele, and uterine descent are significant late complications of the Burch colposuspension procedure in our series. The only preoperative risk factor identified is marked anterior vaginal wall prolapse, and methods to prevent postoperative prolapse remain unclear. We suggest that preoperatively patients be carefully evaluated for genital prolapse. If significant genital prolapse is present, it should be corrected at the time of colposuspension. In patients with a large preoperative cystocele, prophylactic culdoplasty and correction of minimal uterine descent and rectocele should be considered. However, all patients are at risk for the development of postcolposuspension uterovaginal prolapse. During preoperative patient consultations, the risk of postoperative prolapse after the Burch procedure and the possible need for subsequent corrective surgery should be discussed.

We thank Dr. William Graves for his statistical consultation.

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Discussion

Dr. Stewart J. Wetchler, Newport News, Virginia. When an operation is performed to correct stress incontinence, the surgeon considers whether other procedures should be performed concomitantly. Most gynecologists will correct other detectable symptomatic defects in pelvic support. But should asymptomatic defects be corrected? Should any prophylactic procedures be performed to preclude the development of new defects, and are those prophylactic procedures effective? To begin answering these questions, one must know the actual incidence of genital prolapse after surgery to correct stress urinary incontinence. The authors have reported that incidence in this series from a very well respected service.

Wiskind et al. reviewed 131 cases of Burch colposuspension for stress urinary incontinence. They analyzed the need for subsequent surgery for genital prolapse. Of the 131 patients, 35 (or 27%) required 40 additional operations. Thorough analysis demonstrated that the presence of a marked cystocele was the only preoperative finding that predicted the development of postoperative relaxation.

While reviewing this paper, a few questions arise about the study design. First, how were patients selected for a second operation? Was the surgery performed only if symptoms were present or were asymptomatic defects repaired? Second, what were the criteria for "cure" of stress incontinence? The stated cure rate of 81.1% overall is slightly lower than quoted elsewhere, but this may simply reflect stringent criteria for cure. A third question is how many of the patients were menopausal? It is surprising that only three patients were on hormone replacement therapy. If a significant number of postmenopausal patients were included in this study and very few were on hormonal replacement, then their symptoms of genital prolapse might have been exacerbated by urogenital atrophy. Estrogens, of course, will not correct an anatomic defect, but hormone replacement might have relieved symptoms and so decreased the need for surgery to correct genital prolapse. The final question for Dr. Wiskind is how were patients examined preoperatively? Pelvic examinations are usually performed in the supine position. Dr. David Nichols and others have advocated performing examinations in the standing position. Such an examination is awkward to perform, but might detect more defects in support which could be corrected at the initial operation.

The authors state that if a large cystocele is present, "prophylactic culdoplasty and correction of minimal uterine descent and rectocele should be considered." The authors feel, however, that "there may be little that can be done to prevent postoperative prolapse from developing." It is suggested that the addition of a culdoplasty would not decrease the incidence of postoperative enterocele formation and that Dr. Burch had the same experience. In the original 1961 article Burch reported the occurrence of four enteroceles; however, three were after "plication of the cardinal and uterosacral ligaments." An actual culdoplasty was not performed. He goes on to say, "Since amending the plication of the cardinal and uterosacral ligaments and utilizing the Moschcowitz type of procedure the postoperative results have been much better and I do not believe that the development of a postoperative enterocele will be a major problem in the future."[1]

In 1968 Burch still strongly favored cul-de-sac obliteration, and he diagrammed a detailed closure of the pouch of Douglas.[2] Unfortunately, data do not exist to evaluate the effectiveness of such a procedure. The current paper reports a significant 27% incidence of postoperative genital prolapse. It therefore seems prudent to perform any reasonable procedure that should obviate the need for a repeat operation. At the time of retropubic urethropexy, I will therefore continue to perform prophylactic culdoplasty with permanent su-

ture and to correct other significant defects in pelvic support. In patients with severe medical problems, I will minimize anesthetic time and intraoperative risks by only correcting symptomatic or severe defects.

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DR. WILLIAM T. MIXSON, Miami, Florida. I'm disturbed about the definition of mild prolapse; later you said minimal prolapse. That sounds about like the amount of mobility that is present in most parous patients. It is usually asymptomatic and not worthy of consideration either preoperatively or postoperatively. Why can't you leave those patients alone? If you could break the paper down into those who had true prolapse and leave out those that you define as mild prolapse, I think it would have more significance.

DR. WISKIND (Closing). I agree with your point, Dr. Mixson, and during our analysis we were much more concerned with those patients who had marked prolapse as opposed to those who had mild or minimal prolapse. However, mild prolapse was sometimes clinically important because there were patients who had symptomatic mild prolapse that needed to be corrected subsequent to the colposuspension.

As for how the patients were selected for postoperative surgery, most commonly it was by symptoms. Mr. Stanton believes that patients who have asymptomatic prolapse, unless the prolapse was quite significant, should be left alone due to the potential for scarring. Therefore the criteria for postoperative correction of prolapse was the patient's symptoms of pelvic pressure, back pain, or in the case of rectocele, the need for manual assistance during defecation.

The criteria for the cure for stress incontinence was both subjective and objective. First, whether the patient clinically said she was dry; second, the objective visualization of stress incontinence on examination; and third using a modified pad test, where the patients who came back to clinic were asked to do the provocative procedures for the ICS extended pad test while wearing a pad with a piece of nitrazine paper on it to check for urinary leakage. If the patients met all those criteria, they were considered to be dry.

I think that I can comment a little on Mr. Stanton's relatively low cure rate in that St. George's Hospital is a tertiary referral center and many of the patients had previous incontinence surgery before colposuspension.

As far as why so few patients were on hormone replacement therapy, I think that it is a function of the National Health Service in England. They don't advocate putting patients on hormone replacement therapy because it is expensive. The patients have to ask for hormone replacement therapy before they are placed on it. At least that was my experience. It is not encouraged and it is not discussed with patients as they are seen for their clinic visits.

As far as how the patients were examined preoperatively, they were examined with a Sims speculum by Mr. Stanton with the patients on their side. The Sims speculum was first placed against the posterior vaginal wall to look for anterior prolapse and then on the anterior vaginal wall to look for vault and posterior vaginal wall prolapse. Occasionally, the patients were examined erect, but that was certainly not the norm.

Evaluation of pretreatment transvaginal ultrasonography in the management of patients with endometrial carcinoma

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OBJECTIVE: The objective of this study was to evaluate the usefulness of pretreatment assessment with ultrasonography in patients with endometrial carcinoma.

STUDY DESIGN: Fifty patients with endometrial carcinoma diagnosed by endometrial biopsy or curettage were studied before hysterectomy, bilateral salpingo-oophorectomy, and selected lymph node sampling. Ultrasonographic criteria for determination of tumor grade, depth of myometrial invasion, uterine volume, and tumor volume were established. Tumor grade was compared with preoperative and postoperative pathologic diagnoses, depth of invasion was compared with postoperative pathologic evaluation, and uterine volume and tumor volume were compared with postoperative evaluation of tumor grade and depth of invasion. Data were analyzed by χ^2 testing, and, where appropriate, sensitivity, specificity, and accuracy of the ultrasonographic measurements were determined.

RESULTS: The following statistically significant correlations ($p \leq 0.05$) were found. Ultrasonography predicted tumor grade as accurately as preoperative pathologic evaluation did. Depth of invasion ≤50% or >50% significantly correlated. Uterine volume ≤200 ml predicted ≤50% depth of invasion 28 of 32 times. Uterine volume >500 ml was noted in six cases; five of these had grade 2 or 3 tumor and four had depth of invasion >50%. Tumor volume ≤20 ml predicted a grade 1 tumor in 25 of 39 cases and depth of invasion ≤50% in 34 of 39 cases Tumor volume >20 ml correlated with a grade 2 or 3 tumor in 11 of 11 cases and a depth of invasion >50% in seven of 11 cases. In 25 grade 1 tumors diagnosed postoperatively by pathologic study, none was associated with a tumor volume >20 ml.

CONCLUSION: Pretreatment ultrasonographic measurements should be of value in the management of patients with endometrial carcinoma. (Am J Obstet Gynecol 1992;167:406-12.)

Key words: Pretreatment, transvaginal ultrasonography, endometrial carcinoma

Endometrial carcinoma is the most common malignancy of the female reproductive tract. Appropriate initial management is determined by correct preoperative clinical staging; however, the preoperative clinical staging is often incorrect when compared with intraoperative and postoperative pathologic findings.[1-3] We hypothesize that ultrasonographic knowledge of tumor differentiation, depth of myometrial invasion, uterine size, and tumor volume may be useful in making appropriate preoperative planning. The need for more sophisticated evaluation of potential distant metastasis or nodal disease with computerized tomographic (CT) scanning[4, 5] or magnetic resonance imaging (MRI)[6] could be identified. This knowledge might influence clinical management. The use of preoperative radiation[7] or more or less invasive surgical procedures might be indicated.

The evaluation of pretreatment transvaginal ultrasonography and its potential usefulness in the management of patients with endometrial carcinoma is presented.

Material and methods

Fifty-six patients with a diagnosis of endometrial carcinoma by endometrial biopsy or curettage were referred to the ultrasonography unit. Six patients were excluded from the study. Two patients evaluated by ultrasonography were thought to have endometrial hyperplasia (confirmed by a repeat curettage), two patients received radiation therapy, one patient refused surgery, and one patient died before surgery.

After ultrasonographic evaluation all patients had a total abdominal hysterectomy with bilateral salpingo-oophorectomy with selected pelvic or periaortic lymph node sampling.

All ultrasonographic examinations were performed by the same investigator (CFS) with an ATL Ultramark IV with a 5 and 7.5 MHz transvaginal transducer without knowledge of the tumor description on the pre-

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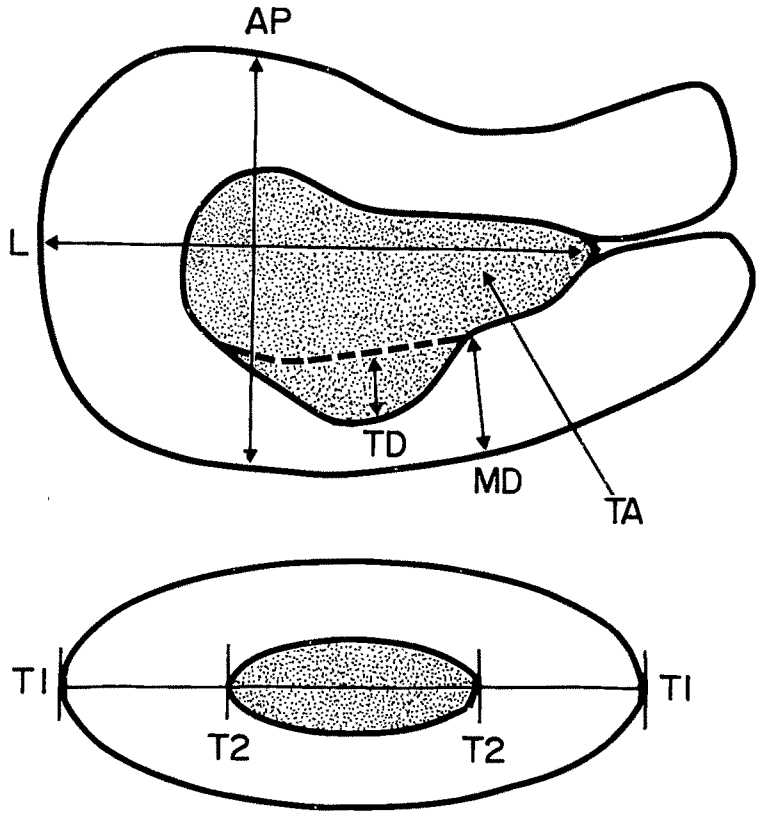


Fig. 1. Uterine measurements: *L*, Longitudinal diameter; *AP*, anteroposterior diameter; *TI*, transverse diameter. Tumor measurements: *TA*, Tumor area; *TD*, tumor depth; *MD*, myometrial depth. Depth of invasion (percent), *TD/MD* × 100; *T2*, transverse diameter.

operative pathology reports. All patients had the following evaluations.

Tumor grade was determined at ultrasonography with the following criteria: grade 1, hypoechoic appearance (relative to myometrium) with a complex mottled, cribriform pattern throughout the tumor; grade 2, hypoechoic appearance with a more homogeneously solid pattern; and grade 3, relatively isoechoic or hyperechoic appearance and a homogeneously solid tumor.

Tumor grade was determined in the preoperative and postoperative pathologic specimens by the Department of Pathology with the recommendations of the International Federation of Gynecologists and Obstetricians.

Depth of myometrial invasion was determined by measuring the distance from the endometrial cavity luminal interface with the myometrium to the most distal depths of tumor, dividing this distance by the total thickness of the myometrial wall involved and multiplying by 100.[8, 9] This is shown in Fig. 1.

Uterine measurements consisted of longitudinal length from the internal os to the outer serosal edge of the fundus in the sagittal plane. An anteroposterior diameter was obtained in the same sagittal view of the greatest diameter of the fundus 90 degrees to the longitudinal axis. The uterine transverse diameter was obtained at the largest diameter in the fundus in the transverse plane. These are illustrated in Fig. 1.

Uterine volume was calculated as a parallelogram by multiplying longitudinal length × anteroposterior diameter × transverse diameter, which is expressed in milliliters. Although this does not represent a true uterine volume because the uterine shape was not taken into account, the measurement of this volume (parallelogram) is approximately 1.6 times the volume of its enclosed uterus.[10]

Tumor measurements consisted of (1) a circumscribed area (square centimeters) of the tumor as viewed in its greatest dimension longitudinally and in a sagittal plane and (2) the transverse measurement of the tumor at its greatest distance in a transverse plane. These are also demonstrated in Fig. 1. Tumor volume was calculated by multiplying the circumscribed area (square centimeters) by the greatest distance in a transverse plane in centimeters; it is expressed in milliliters.

Table IA. Comparison of tumor grade: Ultrasonographic versus preoperative pathologic diagnosis

Ultrasonographic diagnosis	*Preoperative diagnosis*			χ^2	*p Value*
	Grade 1	*Grade 2*	*Grade 3*		
Grade 1	21	4	1		
Grade 2	1	9	2	43.2	<0.001
Grade 3	1	2	9		

Table IB. Comparison of tumor grade: Ultrasonographic versus postoperative pathologic diagnosis

Ultrasonographic diagnosis	*Postoperative diagnosis*			χ^2	*p Value*
	Grade 1	*Grade 2*	*Grade 3*		
Grade 1	24	2	0		
Grade 2	1	9	2	63.7	<0.001
Grade 3	0	1	11		

Table IC. Comparison of tumor grade: Preoperative versus postoperative pathologic diagnosis

Preoperative diagnosis	*Postoperative diagnosis*			χ^2	*p Value*
	Grade 1	*Grade 2*	*Grade 3*		
Grade 1	21	1	1		
Grade 2	3	10	2	51.3	<0.001
Grade 3	1	1	10		

Again, this volume does not represent the true tumor volume but is thought to be consistently related to tumor volume. The true volume can only be calculated when the exact, three-dimensional shape of the tumor is taken into account.

Statistical significance was calculated with the χ^2 test. When appropriate, sensitivity, specificity, and accuracy were determined with the following formulas: Sensitivity = True positive ÷ (True positive + False negative); Specificity = True negative ÷ (True negative + False positive); Accuracy = (True positive + True negative) ÷ (True positive + True negative + False positive + False negative).

Results

The age (mean ± 1 SD) of the study population was 63 ± 14 years with a range of 26 to 83 years. The body weight (mean ± 1 SD) was 185 ± 55 pounds with a range of 108 to 310 pounds. Nineteen (38%) of the patients weighed >200 pounds. The mean time between curettage or endometrial biopsy and ultrasonographic evaluation was 31 ± 16 days with a range of 6 to 71 days. The mean time between ultrasonographic evaluation and hysterectomy was 23 ± 22 days with a range of 1 to 150 days.

After hysterectomy, a diagnosis of endometrial carcinoma was confirmed in 44 patients. Six patients had malignant müllerian mixed tumors, all of which were poorly differentiated (grade 3). The grading of müllerian mixed tumors was based on their most malignant component.

Comparisons of tumor grade by ultrasonography and the preoperative and postoperative pathologic evaluations are presented in Tables IA, IB, and IC. As can be seen, there is good correlation between each pair of grading methods.

Table II compares the depth of invasion as determined by ultrasonography with the postoperative pathologic evaluation. Again, a significant correlation is found between these two measurements.

The relationship between uterine volume and tumor grade is shown in Table III. We examined three critical measurements of uterine volume, i.e., ≤100 or >100, ≤200 or >200, ≤500 or >500 ml. The only statistically significant correlation was found at critical uterine volume of 500 ml.

Table IV depicts the relationship between measurements of uterine volume and depth of invasion. There is a statistically significant correlation between these two measurements at the critical uterine volumes of 200 and 500 ml.

It can be seen in Tables V and VI that tumor volume is statistically significantly related to both tumor grade and depth of invasion. Three critical levels of tumor

Table II. Comparison of depth of invasion: Ultrasonographic versus postoperative pathologic diagnosis

Ultrasonography	*Postoperative pathologic diagnosis*		χ^2	*p Value*
	≤50%	*>50%*		
≤50%	35	5	14.5	<0.001
>50%	3	7		

Sensitivity 58%; Specificity 92%; accuracy 84%.

Table III. Relationship between uterine volume and postoperative pathologic tumor grade

Uterine volume (ml)	*Tumor grade*			χ^2	*p Value*
	Grade 1	*Grade 2*	*Grade 3*		
≤100	10	4	1	4.3	NS
>100	15	8	12		
≤200	19	8	5	5.3	NS
>200	6	4	8		
≤500	24	11	9	6.0	<0.05
>500	1	1	4		

NS, Not significant at $p = 0.05$ level.

Table IV. Relationship between uterine volume and postoperative pathologic depth of invasion

Uterine volume (ml)	*Depth of invasion*		χ^2	*p Value*	*Sensitivity*	*Specificity*	*Accuracy*
	≤50%	*>50%*					
≤100	14	1	3.5	NS	92%	37%	50%
>100	24	11					
≤200	28	4	6.4	<0.05	67%	74%	72%
>200	10	8					
≤500	36	8	6.8	<0.01	33%	95%	80%
>500	2	4					

NS, Not significant at $p = 0.05$ level.

volume were evaluated, i.e., ≤10 or >10, ≤20 or >20, ≤50 or >50 ml. Statistical significance was found at all three critical levels.

Comment

We have attempted to determine the practical usefulness of pretreatment measurements of tumor grade, depth of invasion, uterine volume, and tumor volume by ultrasonography.

Without ultrasonography (or some other imaging technique), the only information available to the gynecologic surgeon is uterine size by physical examination and tumor grade by endometrial biopsy or curettage. Because many of these patients are obese, viewing the uterus with ultrasonography should improve the estimation of uterine size. In addition, evaluation of depth of invasion and tumor volume are not available by standard procedures. Tumor extension into the cervix and extension beyond the uterus, although not presented here, have been described by other investigators.[11]

Comparing the accuracy of preoperative pathologic evaluation with ultrasonography in predicting the postoperative pathologic tumor grade, the postoperative pathologic evaluation showed grade 1, 2, or 3 tumors in 25, 12, and 13 patients, respectively. The preoperative pathologic evaluation correctly predicted grade 1, 2, and 3 tumors in 84%, 83%, and 77% of patients, respectively. The overall accuracy was 82%. Grading by ultrasonography was correct in 96%, 75%, and 85% of the patients, respectively. Overall accuracy was 88%. Another way of evaluating these data (probably a more useful way clinically) is to determine the accuracy of the predictions. The preoperative pathology report showed 23 grade 1, 15 grade 2, and 12 grade 3 tumors. The preoperative reports were correct 91%, 67%, and 83% of the time. Overall predictive accuracy was 82%. At prospective ultrasonographic evaluation, there were 26 grade 1, 12 grade 2, and 12 grade 3 tumors, respectively. The evaluations were correct 92%, 75%, and 92% of the time. Overall accuracy was 88%. It would appear that tumor grading by ultrasonography is at

Table V. Relationship between tumor volume and postoperative pathologic tumor grade

Tumor volume (ml)	*Tumor grade*			χ^2	*p Value*
	Grade 1	*Grade 2*	*Grade 3*		
≤10	19	5	3	10.6	<0.01
>10	6	7	10		
≤20	25	8	6	9.6	<0.01
>20	0	4	7		
≤50	25	10	9	8.0	<0.05
>50	0	2	4		

Table VI. Relationship between tumor volume and postoperative pathologic depth of invasion

Tumor volume (ml)	*Depth of invasion*		χ^2	*p Value*	*Sensitivity*	*Specificity*	*Accuracy*
	≤50%	*>50%*					
≤10	25	2	8.9	<0.01	83%	66%	70%
>10	13	10					
≤20	34	5	14.2	<0.001	58%	89%	82%
>20	4	7					
≤50	36	8	6.8	<0.01	33%	95%	80%
>50	2	4					

least as accurate as grading by preoperative endometrial biopsy or curettage.

Knowledge of depth of invasion of the tumor into the myometrial wall is additional information available by ultrasonography not available by biopsy or curettage. When ultrasonography evaluation predicted depth of invasion ≤50% or >50%, the predictions were correct 88% and 70% of the time, respectively. This accuracy is comparable with more sophisticated imaging techniques.[11, 12]

With regard to the measurement of uterine volume, it would appear that knowledge of small (≤200 ml) or large (>500 ml) uterine volumes may be useful as predictive values. A uterine volume ≤200 ml predicted ≤50% depth of invasion 88% of the time. A uterine volume >500 ml predicted a grade 2 or 3 tumor and >50% depth of invasion 83% and 67% of the time, respectively. Although knowledge of large uterine volume (>500 ml) would appear to provide the more useful information, these volumes were found in only 12% of study patients.

Tumor volume ≤20 or >20 ml may be of predictive value. Tumor volumes ≤20 ml correctly predicted a grade 1 tumor and ≤50% depth of invasion 64% and 87% of the time, respectively. A tumor volume >20 ml predicted a grade 2 or 3 tumor and >50% depth of invasion 100% and 64% of the time, respectively. In none of 25 cases was a grade 1 tumor associated with a tumor volume >20 ml.

We conclude that these data support the hypothesis that pretreatment ultrasonographic measurements may provide useful information before surgery. The gynecologic oncologist might decide that further studies, such as MRI, are indicated or may modify the treatment plan to include radiation therapy or a more or less extensive surgical procedure.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

DR. MARK A. CROZIER, Greenville, South Carolina. No aspect of gynecologic oncology is more confusing and yet more relevant to obstetricians and gynecologists than the management of endometrial carcinoma. There is considerable variation in the type of surgery recommended; which, if any, adjuvant treatment is needed; and the timing of the adjuvant therapy. An attempt to clarify this confusion is not the purpose of this paper. What is well established is that there are patients with well differentiated cancers and superficial myometrial invasion who have a very low incidence of retroperitoneal nodal metastasis and survival rates around 90% that can be adequately treated with total abdominal hysterectomy and bilateral salpingo-oophorectomy without extended surgical staging or adjuvant irradiation. Patients with poorly differentiated or deeply invasive tumors have a higher incidence of nodal metastasis and poorer survival rates and probably should have adjuvant therapy or extended surgical staging.

Most patients with endometrial cancer are initially seen by or referred to an obstetrician or gynecologist. Few will be directly seen by a gynecologic oncologist. Most obstetricians and gynecologists in general practice are not comfortable performing the extended surgical staging currently recommended for endometrial carcinomas. However, a significant percentage of endometrial cancers are well-differentiated, superficial cancers that can be adequately treated with total abdominal hysterectomy and bilateral salpingo-oophorectomy without extended surgical staging or adjuvant therapy. Referral to a gynecologic oncologist results in additional expense, perhaps referral out of the patient's home community, and loss of continuity of care with the primary physician. In addition, many of these patients are elderly, obese, or have medical problems and can ill afford extended surgery or adjuvant irradiation therapy that will not benefit them. Several studies have established the relative inaccuracy of clinical staging, i.e., physical examination and curettage. This is one of the reasons that International Federation of Gynecologists and Obstetricians has adopted a surgical pathologic method of staging endometrial carcinomas. An accurate preoperative method of assessing prognostic factors such as grade and depth of myometrial invasion would allow the patients with well-differentiated superficial cancers to be treated by total abdominal hysterectomy and bilateral salpingo-oophorectomy without referral, while those with poorly differentiated or deeply invasive cancers could be referred for extended surgical staging or adjuvant therapy. This study has attempted to determine the ability of preoperative vaginal probe ultrasonography to predict two well-known prognostic factors in endometrial carcinoma, that is, final pathologic grade and depth of myometrial invasion. It has also introduced two ultrasonographic parameters that seem to correlate with grade and depth of invasion, that is, uterine volume and tumor volume.

Pretreatment vaginal probe ultrasonography predicted postoperative pathologic grade in 24 of 26 grade 1 tumors, nine of 12 grade 2, and 11 of 12 grade 3 tumors. All of these were statistically significant correlations. Preoperative vaginal probe ultrasonography predicted postoperative depth of invasion for 35 of 40 $\leq$50% and seven of 10 >50% invasion. Both of these achieved statistical significance. Tumor volume showed statistical correlation with both grade and depth of invasion. Of note was that no grade 1 cancer had a tumor volume >20 ml and that 11 of 11 tumors >20ml were grade 2 or 3. The numbers are small, but vaginal probe ultrasonography appears to predict, with a high degree of accuracy, both grade and depth of invasion. Combining this with the parameters of uterine volume and tumor volume, we may be able to accurately predict preoperatively significant pathologic prognostic factors and provide more effective and less morbid treatment.

I would like to close by asking several questions. First, have there been comparisons between vaginal probe ultrasonography, CT scan, and MRI in predicting grade and depth of invasion? Second, is there any reason to order an MRI if a vaginal probe ultrasonographic examination is done? Third, have tumor volume and uterine volume been looked at as independent prognostic factors in endometrial cancer? Finally, does uterine volume add anything that tumor volume won't tell you?

DR. CHARLES F. SHIPLEY III (Closing). Dr. Crozier has certainly identified several applications of ultrasonography in its use in patients with endometrial carcinoma. I would first like to address his questions individually. "Have there been comparisons between vaginal probe ultrasonography, CT scan, and MRI in predicting grade and depth of invasion?" I know of no study where all three techniques were performed on the same patient sample with direct comparisons made of the predictive accuracy of the methods. However, there are certainly several studies that have analyzed individual techniques for predicting grade and depth of invasion. Although I have not analyzed or compared them statistically, they certainly do not appear to be very similar in their predictions.

The second question was, "Is there any reason to order an MRI if vaginal probe ultrasonography is done?" If the history, physical examination, pathology reports, and ultrasonographic data collectively suggest a very good possibility of nodal disease or distant disease, then I certainly think CT and MRI may be helpful

and appropriate to evaluate the process. The corollary is also true; if the data strongly suggest that the tumor is limited to the uterus, then I suspect that CT and MRI would not be necessary.

Third, "Have tumor volume and uterine volume been studied as independent prognostic factors in endometrial carcinoma?" To my knowledge, tumor volume has not previously been evaluated as a prognostic factor in endometrial carcinoma transvaginally. Uterine size classically has been evaluated by measuring the depth of the uterine cavity in the past. I strongly suspect that this was a very crude attempt to define tumor volume as opposed to actual uterine size. I know of no study in which the evaluation of uterine size by measuring its volume has been adequately evaluated.

Finally, "Does uterine volume add anything to tumor volume that tumor volume won't tell you?" First let me say that the determination of uterine volume, as you can see from the presentation, is a very easy measurement to make and not extremely time consuming. It seems to me that the best way to use this measurement would be when the uterus is either very small or very large. Small uteri were associated with very, if you will, low risk or less aggressive disease and large uteri are associated with more severe disease. It simply contributes additional information when trying to differentiate between mild and severe disease. It also is very difficult in very large uteri to evaluate the endometrial cavity because of changes in the distance to the endometrial cavity where the resolution is not quite as good; therefore it may be helpful. It is my belief that the measurement of tumor volume will eventually be shown to be a more useful measurement; however, I see no reason to discontinue making the measurement at this time.

The treatment of pelvic soft tissue radiation necrosis with hyperbaric oxygen

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OBJECTIVE: The purpose of this study was to examine the therapeutic effects of hyperbaric oxygen on radiation-induced soft tissue necrosis in patients who previously received treatment for a gynecologic malignancy.

STUDY DESIGN: Fourteen patients whose necrotic wounds failed to heal after 3 months of conservative therapy were enrolled in a prospective observational study. Outcome of hyperbaric oxygen treatment was measured by resolution of necrosis.

RESULTS: Fourteen patients underwent 15 courses of hyperbaric oxygen treatments. All those with radiation necrosis of the vagina alone or in association with rectovaginal fistula had complete resolution of necrosis with hyperbaric oxygen. Only one treatment failure occurred.

CONCLUSION: Hyperbaric oxygen is a safe, effective, and well-tolerated treatment for radiation-induced soft tissue necrosis. (Am J Obstet Gynecol 1992;167:412-6.)

Key words: Soft tissue radiation necrosis, vaginal necrosis, rectovaginal fistula, hyperbaric oxygen

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The efficacy of radiation therapy in the management of malignant disease is well established. Adequate tumoricidal doses may result in adverse effects on surrounding healthy tissue. The pathophysiologic characteristic of radiation tissue damage is one of a progressive obliterative endarteritis, leading to a hypovascular, hypoxic, and hypocellular tissue bed.[1]

Subsequent tissue breakdown may occur spontaneously, particularly when the total radiation dose is high or as a result of direct trauma. The greatly increased demand for oxygen and other cellular metabolites associated with tissue healing will exceed available supply in this setting, leading to a chronic, nonresolving wound. Although the sensitivity to radiation damage varies from tissue to tissue, therapeutic radiation-induced necrosis has been reported in a wide variety of anatomic sites. Hyperbaric oxygen therapy has recently been demonstrated to overcome the effects of late radiation-induced changes by a potent angiogenic effect. Intermittent exposure to elevated oxygen doses at 2.0 to 2.5 atmospheres absolute pressure will effectively manage tissue hypoxia and reestablish the oxygen gradient necessary to induce wound healing responses.[2] The majority of clinical experience has centered on the treatment of osteoradionecrosis of the mandible with limited experience involving soft tissue, bladder, myelin, and gastrointestinal tract.[3,4] This article documents the successful use of hyperbaric oxygen therapy in the management of radiation damage to the cervix and vaginal vault.

Material and methods

Patients referred from the Division of Gynecologic Oncology at the University of South Carolina School of Medicine to the Department of Hyperbaric Medicine at Richland Memorial Hospital for treatment of radiation-induced soft tissue necrosis were studied. Patients were examined by senior faculty, and aggressive attempts were made to rule out recurrent or metastatic disease with routine radiographic procedures and biopsy of necrotic wounds.

Patients accepted for hyperbaric oxygen therapy were free of active malignancy for at least 6 weeks before beginning treatment. Patients also had failure of healing after 3 months of conservative therapy. This therapy included hydrogen peroxide or antiseptic irrigation, topical conjugated estrogen, nutritional supplementation, and surgical debridement where indicated.

On referral all patients were examined by a hyperbaric medicine consultant. A thorough history was obtained and physical examination was performed to ensure that no medical contraindications to hyperbaric oxygen therapy were present. A course of therapy was then outlined to include number of treatments, total duration of each treatment, and number of treatments per day.

A Sechrist Monoplace (Sechrist Industries, Anaheim, Calif.) hyperbaric chamber was used for all patients. Compression to 2.0 atmospheres absolute pressure was undertaken while the patient breathed 100% oxygen. Patients were carefully monitored during each treatment session by registered nurses under the supervision of the Director of Hyperbaric Medicine.

Patients were examined by the Gynecologic Oncology faculty at the end of the recommended course of therapy, and results of treatment were documented.

Results

From 1986 to 1991, 18 patients with radiation-induced soft tissue necrosis were referred by Division of Gynecologic Oncology for hyperbaric oxygen therapy. Four patients did not complete therapy. Therapy was discontinued in three with histologic evidence of recurrent disease. A fourth patient refused to continue therapy because of severe anxiety associated with confinement in the hyperbaric chamber. Fourteen patients completed 15 courses of the recommended number of treatments. The mean age of the patients was 53 years, with a range from 35 to 78 years. The primary site of cancer was cervix (nine patients), endometrium (one patient), vagina (three patients), and colon (one patient). All patients had received external beam radiation with a mean dose of 4830 Gy. In addition, 53% of patients received intracavitary radiation.

Eleven patients received hyperbaric oxygen therapy for soft tissue radionecrosis of the vaginal vault alone. Two patients were treated for vaginal necrosis with an associated rectovaginal fistula. One of these patients had previously been successfully treated with hyperbaric oxygen therapy for soft tissue radionecrosis of the vagina 4 years before development of recurrent necrosis of the vagina with rectovaginal fistula formation. One patient was treated for necrosis of the abdominal wall in the area of external beam radiation therapy. Another patient developed massive necrosis of the abdominal wall, sacrum, and vagina with development of large vesicovaginal and rectovaginal fistulas.

Hyperbaric oxygen therapy was given at 2.0 atmospheres absolute pressure one to two times per day for 90 to 120 minutes each. The average number of treatments was 44 with a range of 18 to 143. Compression was well tolerated in all patients. The only complication was seen in one patient who suffered a ruptured tympanum, which healed spontaneously without sequelae. No patient required interruption of therapy due to a complication of compression.

Each of the 11 patients with soft tissue radionecrosis of the vagina alone showed marked improvement or complete resolution of necrosis at the completion of therapy. Complete resolution was seen in all patients within 8 weeks of hyperbaric oxygen therapy. One of these patients developed recurrent squamous cell carcinoma of the cervix in the bladder base. She underwent anterior exenteration one year after hyperbaric oxygen therapy. The vaginal cuff healed well, and there is no evidence of necrosis 9 months after surgery. Both

patients with rectovaginal fistulas underwent fecal diversion by loop colostomy followed by hyperbaric oxygen therapy with resolution of necrosis. Rectovaginal fistula repair was performed with subsequent closure of loop colostomy. Three years after surgery there is no evidence of recurrent necrosis or fistula. A patient with necrosis of the panniculus had complete healing after hyperbaric oxygen therapy.

The only treatment failure occurred in one patient who developed massive necrosis of the abdominal wall, sacrum, and vagina with rectovaginal and vesicovaginal fistula formation. She underwent urinary and fecal diversion, repetitive surgical debridement, skin grafting, aggressive wound care, nutritional support, and intensive hyperbaric oxygen therapy totaling 143 treatments. Bacterial overgrowth complicated wound healing and the necrosis progressed, eventually leading to her death.

Comment

The goal of radiation therapy is to deliver tumoricidal doses with minimum possible adverse effects on adjacent healthy tissue. In recent years a better understanding of radiobiologic principles has served to reduce the degree of iatrogenic damage through the establishment of upper radiation limits, fractionated exposure protocols, and the development of high-energy radiation delivery systems.

In spite of these improvements, localized radiation-induced tissue breakdown continues to present a clinical challenge. The most common form of this disease is osteoradionecrosis of the mandible, although it has been reported in a wide variety of other bony and soft tissue sites.[3-5] The pathophysiologic features of localized radiation tissue injury have recently been redefined.[1] The long accepted and empirically derived sequence of radiation, trauma, and infection has been replaced by a description that more accurately describes the biochemical and cellular pathology. Histologic analysis of tissue biopsies from 58 patients revealed the postradiation, iatrogenic process as one of a progressive, obliterative endarteritis. This process results in a decrease in blood flow, leading to a hypovascular, hypoxic, and hypocellular tissue bed.

Another misunderstanding of the pathologic process of radiation tissue injury has been that revascularization would gradually occur with time. Therefore it was thought that any surgical procedures contemplated in this area should be delayed as long as possible. Marx[6] has demonstrated that as time passes there is less vascularity, less tissue perfusion, and more fibrosis and greater risk for a nonhealing surgical site.

The treatment of local tissue breakdown as a secondary effect of radiation therapy is a frustrating medical and surgical dilemma. Hypovascularity predisposes to tissue breakdown, either spontaneously or after direct trauma. Radiation-induced ulcerations are painful, and adequate control of pain without addicting medications is often impossible. Surgical incision of the obvious lesion may lead to the development of a larger nonhealing wound because adjacent tissue is also compromised. Attempts to cover external wounds with skin grafts or flaps typically requires repeated procedures and, in many cases, still proves unsuccessful.

About 10% of all patients treated with radiation to the pelvis for a gynecologic malignancy will develop a superficial vaginal vault necrosis that may become a progressive problem predisposing to fistula formation. Traditional therapy of vaginal vault necrosis consists of hydrogen peroxide douches, sodium hypochlorite douches, and estrogen cream.[7-9] If fistulas develop, fecal or urinary diversion is required. Fistula repair, when attempted, is complicated by the hypovascularity and tissue hypoxia present in the irradiated vagina.

During the 1970s hyperbaric oxygen therapy was introduced for the management of radiation-induced tissue damage.[10, 11] The subacute and chronic phases of the radiation wound are particularly suited to this form of therapy. Hyperbaric oxygen acts to stimulate collagen formation at the wound edges through elevation of local tissue oxygen tensions.[2] New microvasculature dependent on a collagen matrix for physical support is greatly enhanced in this setting and allows reepithelialization to occur. A 20 to 30 day course of hyperbaric oxygen will provide a rich capillary bed in surrounding radiation-damaged tissue that, although hypoperfused and hypoxic, is still viable. This angiogenic effect is clearly dependent on the administration of oxygen under hyperbaric conditions. A recent comparative study failed to demonstrate such revascularization when oxygen was administered under normobaric conditions, in contrast to the effect when oxygen was administered under hyperbaric conditions.[12]

In 1977 Glassburn and Brady[13] reported on 27 patients who received hyperbaric oxygen therapy for necrotic wounds. Twelve patients were treated for necrosis of the vagina or perineum. All eight of the patients who completed therapy showed marked improvement or complete healing of their wounds. The only complication was rupture of a tympanic membrane.

Hyperbaric oxygen therapy for radiation-induced vaginal vault necrosis is safe and well tolerated by most patients. We believe hyperbaric oxygen therapy to be an effective method for promoting healing in areas of radiation necrosis. The effects of this healing improves patient comfort and may also prevent the more serious problem of fistula formation. In those patients with rectovaginal fistula, hyperbaric oxygen therapy after fecal diversion may decrease the morbidity and increase the success of fistula repair.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

Dr. W. Allen Addison, Durham, North Carolina. Regional necrosis induced by radiation directed toward the cure of cancer is a difficult and frustrating problem. Frequently the necessary concern about the possibility of uneradicated malignancy leads to the repeated trauma of biopsies in tissue already so compromised as to defy healing. Such biopsies, and possibly local measures meant to cleanse and debride, may well serve to aggravate necrosis in these fragile and sorely devitalized tissues.

I suspect that all services that treat gynecologic malignancy by radiation, and which have access to hyperbaric oxygen therapy, have put patients like those who constitute the subject of this report into the hyperbaric chamber. Our own experience with such patients has been mixed and includes both apparent dramatic successes and clearly dismal failures.

A Medline search done for the purpose of this discussion revealed that there are no published reports in the gynecologic literature about the use of hyperbaric oxygen in the treatment of radiation-induced necrosis. That fact alone certainly enhances the importance of this report.

Now one might be stringent and point out that in this report radiation-induced soft tissue necrosis is not clearly defined and that no measurements of the areas or volume of necrosis are given. Additionally, one might inquire of the authors precisely what determined the number of hyperbaric oxygen treatments that were given to each patient and why the evolving bacteriology of these necrotic ulcers was not followed during therapy. Or, one might be pragmatic and accept the fact that such a study as this cannot be fairly subjected to the sort of scientific exactitude demanded by maternal-fetal medicine.

It took the authors 5 years to find 14 evaluable patients. These patients had not responded to traditional and less expensive treatment than hyperbaric oxygen, and they were desperate. Their prospects included progressive disability, addiction, and death. In this setting, there are no options for the randomization and controls that would be demanded by a reproductive endocrinologist. The only real option was to pull out all stops, treat these desperate patients as intensively as possible, and observe the results as carefully as possible. This, I believe, is what was done. I for one continue to have faith in the careful observations of seasoned clinicians.

I have checked with experts on the subject at the institution where I work and have been assured that technically the hyperbaric oxygen therapy was administered appropriately. I have also been assured by them that a therapy as expensive as hyperbaric oxygen is not likely to be invoked if there are acceptable alternatives.

My only questions to Dr. Williams are the following: (1) What did determine the number of hyperbaric oxygen treatments a patient received? I assume it was healing of clinically detectable necrosis, but this is not stated in the manuscript. (2) What was the cost of this therapy? and (3) Would earlier institution of hyperbaric oxygen therapy in such patients as those presented arrest the process sooner and result in shorter necessary treatment and less ultimate expense? If so, could patients be identified who should be started on hyperbaric oxygen at the earliest signs of radiation-induced necrosis, such as those with preexisting microvascular disease?

Dr. Williams (Closing). The number of hyperbaric oxygen treatments received by each patient was determined by the hyperbaric medicine consultant. At the beginning of this project there were a larger number of treatments per person than there were toward the end. In 1988 Marks[1] working with Johnson, both dentists skilled in treating carcinomas of the mouth, reported on the measurement of transcapillary oxygen tension in irradiated tissues of the jaw. They described an S-shaped curve of oxygen tensions with three distinct phases. The first, or lag phase, identifies no measurable angiogenesis. This is a phase of collagen synthesis and capillary budding. Oxygen tensions remain approximately 30% that of nonirradiated tissue until the eighth exposure to hyperbaric oxygen. In the sec-

ond, or rapid-rise phase, oxygen tensions increase geometrically to a maximum of 82% to 85% between the eighteenth and twenty-third exposures. During this phase visible sign of angiogenesis are present. In the third or plateau phase the transcapillary oxygen tension values of the irradiated tissues are 80% to 85% of those of nonirradiated tissue. Oxygen tension never reaches the same value as in nonirradiated tissue. These levels do remain stable over several years. On the basis of these data, our current protocol calls for 20 hyperbaric oxygen treatments. At the end of this period the patient is reevaluated and may be referred for 10 to 20 more treatments if there is a good response but one that is felt to be inadequate. This may be particularly important in patients who have larger lesions.

A little bit more about the costs—The average total treatment cost is about $8000. No cost-benefit analysis has been done for the treatment of this particular condition, but extensive work has been done on osteoradionecrosis of the mandible.[2] It has been estimated that conventional therapy for osteoradionecrosis of the mandible costs about $69,000, with a cure rate of around 8%. The treatment of osteoradionecrosis of the mandible with a 100% cure rate costs about $30,000. The prevention of osteoradionecrosis with hyperbaric oxygen in those patients who may undergo surgery in this area is about 95% successful and costs $8000. So we see a significant cost savings in that particular area.

As far as identifying patients who may be at particular risk, our patient population had diseases that may have reflected some microvascular problem. There were a fair number of diabetics and hypertensives. Although we have a much larger number of patients with hypertension and diabetes who don't have development of radiation necrosis, of course, it would be very difficult to predict those patients.

Earlier treatment would be nice, but unfortunately our patients are first seen with this necrosis the same way that they are first seen with their cancers. Usually it is in an advanced stage and we are unable to catch it very early.

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The incidence of cerebral palsy

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The clinician is often held responsible for obstetric events that are suspected of being related to cerebral palsy. To review the incidence of cerebral palsy and to aid the clinician in this situation, a search of published studies was conducted. Composite rates of cerebral palsy in different birth weight infants and cerebral palsy with and without serious mental retardation were calculated. The cumulative incidence rate at the age of 5 to 7 years was 2.7 cases of cerebral palsy for 1000 birth cohorts. Approximately 36% of all cerebral palsy occurred in the infant <2500 gm. Serious mental retardation (intelligence quotient <50) accompanied cerebral palsy approximately 30% of the time for the term infant and 18% of the time when the infant was <2500 gm. On the basis of a past estimation that 70% of cerebral palsy is of antepartum or unknown origin, the term infant at risk for intrapartum-attributed cerebral palsy may be about 1 in 2000 term births. (AM J OBSTET GYNECOL 1992;167:417-23.)

Key words: Cerebral palsy, incidence, mental retardation

Cerebral palsy continues to pose a diagnostic and therapeutic dilemma for the obstetrician because its various causes are poorly understood. Electronic fetal monitoring, initiated in the early 1960s, raised hopes that some cases of cerebral palsy could be prevented through a better means of evaluation of the fetus during labor. Today, studies continue to find a lack of association between intrapartum fetal monitoring and long-term neurologic outcome.[1] Confounding the incidence issue is the fact that obstetric practices and neonatal intensive care have changed. With increased infant survival, increased neurologic morbidity may occur more frequently, offsetting the overall effects of clinical practice changes.[2]

This review attempted to evaluate current morbidity data with respect to the rates of cerebral palsy. Past studies have generally found a prevalence rate of about 2 per 1000 living infants at 7 to 10 years of age, with serious mental retardation (intelligence quotient [IQ] <50) present in 20% to 30% of these children.[3] The largest United States study, the Collaborative Perinatal Project, found a cerebral palsy cumulative incidence rate (which includes children with cerebral palsy who died) of 4.6 per 1000 of infants at 7 years of age.[4] The question raised in this review was whether the cumulative incidence of cerebral palsy has changed during the past 10 years, specifically the cumulative incidence in the term infant.

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6/1/37971

Methods

A Medline search was conducted of all original English language studies published since 1980, with major infant neurologic damage as an end point. Unfortunately, although these studies were published since 1980, the birth dates of the populations often went back to the 1950s. Few recent studies of the combination of mental retardation and cerebral palsy were found; therefore studies conducted before 1980 were included when that aspect is discussed.

Where possible, in some of the tables the birth dates were indicated rather than the publication dates of the studies, so that the reader could consider the possible differences in neonatal care in the different time periods. To be included in analyses, studies had to provide adequate raw numbers to determine the denominator for the rates of cerebral palsy either in the total population or by birth weight. Cumulative incidence at the age of 4 to 7 years was used because it provides more information than either a straight incidence or prevalence rate. The cumulative incidence rate used all births during a set period of time as the denominator, and numerator included infants with cerebral palsy who had died. Including infants who had died has been recommended to provide as complete a picture of cerebral palsy as possible.[5] Prevalence rate was not used,

Table I. Cumulative incidence of cerebral palsy per 1000 live births

Study	*Year published*	*Birth years*	*Country*	*Length of follow-up (yr)*	*Rate, excluding acquired (per 1000)*
Jarvis et al.[9]	1985	1972-1975	England	5	1.8
Nelson and Ellenberg[4]	1986	1959-1966	United States*	7	4.6
Emond et al.[10]	1989	1970	England	7	2.5
Hagberg et al.[11]†	1989	1979-1982	Sweden	4	2.2
Holst et al.[12]	1989	1978	Denmark	4	4.9
Dowding and Barry[13]‡	1988	1979-1981	Ireland	4	1.9
Stanley and Watson[14]	1988	1979-1981	West Australia	5	2.3
Riikonen et al.[15]	1989	1978-1982	Finland	5	2.5
Torfs et al.[2]	1990	1959-1966	California	5	2.0§
Pharoah et al.[17]	1990	1984	England	5	1.9
Meberg[16]	1990	1980-1984	Norway	4	2.1
Composite rate					2.7

*Collaborative Perinatal Project.

†Excluded deaths before age 2.

‡Excluded all congenital anomalies.

§Did not indicate whether deaths were included.

because it can vary tremendously depending on the age of diagnosis and the length of time of survival. In the Collaborative Perinatal Project 15% of the children diagnosed with cerebral palsy at 12 months had died by the age of 7 years.[6, 7]

Studies with follow-up at different ages, between 4 and 7 years, were qualified by stating the different ages. Cases of cerebral palsy that were obviously caused postnatally were excluded. Studies usually did not differentiate cerebral palsy by degree of handicap, so a study's diagnosis of cerebral palsy was accepted. Figures from the Collaborative Perinatal Project were included to provide comparisons and because that study constituted a large and essentially singular United States cerebral palsy incidence study. The figures from the Collaborative Perinatal Project included both infants who had a diagnosis of cerebral palsy who had died and cases of cerebral palsy that they defined as mild (not requiring bracing, wheelchair, etc.)[8] because we decided that their "mild" category would be included in all cases of cerebral palsy in other studies. We primarily used later published reports about the Collaborative Perinatal Project because these categories were included. Earlier reports that excluded deaths and "mild" cerebral palsy yielded a 2.5 per 1000 rate.[8]

For types of cerebral palsy, the denominator is all cases of cerebral palsy. When discussing the different types of cerebral palsy, the most frequently used categories of cerebral palsy included diplegia (or paraplegia), a form of cerebral palsy where the legs are primarily affected; hemiplegia, where only one side of the body is affected; and quadriplegia (or tetraplegia), where three or all four limbs are affected. Extrapyramidal (or dyskinetic) cerebral palsy, including choreoathetotic and dystonic and ataxia, was included as much as possible when studies had this as a distinct category.

The analyses of cerebral palsy by birth weight were done with live births at that birth weight as denominators. Because studies used either cerebral palsy or mental retardation as starting points, both types of studies were considered. However, when considering studies using mental retardation as the starting point, the primary focus was that proportion of the mentally retarded who also had cerebral palsy. Mental retardation not associated with cerebral palsy was not reviewed.

Results

Excluding cases that had an obvious postnatal origin, 10 studies published since 1980 determined the cumulative incidence rates for cerebral palsy[2, 4, 9-17] (Table I). The average cumulative incidence rate of these studies was 2.7 per 1000 livebirths.

Table II describes studies that included the breakdown by type of cerebral palsy.[8, 9, 11, 13, 17-19] Years of birth, not publication, are given. Not all the previously discussed studies were included, because not all had breakdown by type of cerebral palsy. The average proportions of the different types of cerebral palsy of all with cerebral palsy were 34% diplegia, 30% hemiplegia, 20% quadriplegia, and 16% extrapyramidal.

Table III includes studies that grouped cerebral palsy by birth weight.[2, 4, 9, 11-17, 20] Years of birth, rather than dates of publication, are indicated for the reasons previously stated. The average proportions of cerebral palsy by birth weight of all children with cerebral palsy were 36% for those weighing <2500 gm and 64% for children weighing >2499 gm.

Some of these studies analyzed cerebral palsy rates by smaller increments of birth weight[6, 11, 13, 14, 16, 17] (Table IV). The increments varied too much to do an exact comparison, but the figures are helpful to determine any trends in the incidence by birth weight.

Table II. Distribution of cerebral palsy by type*

Study	*Year of birth*	*Country*	*Diplegia (%)*	*Hemiplegia (%)*	*Quadriplegia (%)*	*Extrapyramidal (%)*
Nelson and Ellenberg[8]†	1959-1966	United States	32	29	24	14
Pharoah et al.[17]	1984	England	24	40	27	11
Dowding and Barry[13]	1979-1981	Ireland	38	25	10	27
Dale and Stanley[19]	1971-1975	Australia	39	22	32	5
Riikonen et al.[15]	1978-1982	Finland	44	29	3‡	24
Hagberg et al.[11]	1979-1982	Sweden	41	36	7‡	15
Jarvis et al.[9]	1972-1975	England	18§	29	39	9
Composite rates			34	30	20	16

*Denominators are all cases of cerebral palsy. Rows may not total 100% because some types of cerebral palsy were excluded.
†Based on survivors.
‡Used term *tetraplegia*.
§Used term *paraplegia*.

Table III. Distribution of cerebral palsy by birth weight*

Study	*Year of birth*	*Country*	*<2500 gm (%)*	*>2499 gm (%)*	*Overall low-birth-weight births (%)*
Nelson and Ellenberg[4]	1959-1966	United States†	33	67	9.2
Pharoah et al.[17]	1984	England	47	53	6.7
Stanley and Watson[14]	1979-1981	West Australia	32	68	4.1‡
Holst et al.[12]	1978	Denmark	40	60	3.5
Jarvis et al.[9]	1972-1975	England	26	74	7.0
Meberg[16]	1980-1984	Norway	27	73	4.2
Hagberg et al.[11]	1979-1982	Sweden	38	62	4.0
Dowding and Barry[13]	1979-1981	Ireland	32	68	5.1
Riikonen et al.[15]	1978-1982	Finland	52	49	4.0
Saito et al.[20]	1963-1976	Japan	52	48	4.5
Torfs et al.[2]	1959-1966	California	17	83	6.8
Composite rates			36	64	5.3

*Denominator is cases of cerebral palsy.
†Collaborative Perinatal Project.
‡Figure from Dale et al.[19]

Some studies analyzed trends of cumulative incidences of cerebral palsy over periods of time[9-11, 13-17, 19] (Fig. 1). Some studies found recent minor decreases, others found almost no change in rate, and other studies found recent minor increases. When there was a minor increase, the type of cerebral palsy that increased the most was diplegia or cerebral palsy among low-birth-weight (LBW) infants.

A few studies analyzed the occurrence of serious mental retardation among children with cerebral palsy. Table V provides the figures from those studies.[18, 21, 22] The average rate of serious mental retardation (IQ <50) along with cerebral palsy for all birth weights was 25%, 18% for infants weighing <2500 gm, and 30% for those >2499.

A few studies analyzed the occurrence of cerebral palsy among children with serious mental retardation.[23-28] These figures are based on children surviving at the time of the study (prevalence). The composite rate for the studies of all cases of mental retardation with cerebral palsy was 16.8% (Table VI). McLaren and Bryson[29] reviewed studies for the presence of cerebral palsy in the seriously mentally retarded. They encountered difficulties due to differences in methodology and a frequent lack of differentiation between disorders; for example, neurologic, motor, and sensory disorders may have been combined. Four studies were sufficiently specific enough to be analyzed.[23-26] Kiely[30] analyzed two other studies[27, 28] for the occurrence of cerebral palsy in populations with serious mental retardation. Because of methodologic differences used in the studies, this composite rate should be considered as an approximation.

Comment

One task in standardizing studies was to consider when the follow-up was done and when the diagnosis of brain damage was made. Nelson and Ellenberg[6, 7] pointed out the difficulty of comparing studies because of the variation in timings of follow-up. Studies generally grouped all degrees of cerebral palsy together; however; milder cases often were not known until the child entered school and as many as 50% of patients diagnosed at 12 months were free of motor handicap at 7 years of age. A cumulative incidence rate addresses the problem of loss of cases through deaths.

There was a problem with the lack of data on births that occurred in the past 10 years. The only reviewed

Table IV. Rate of cerebral palsy by birth weight (per 1000)

Study	*Rate in infants <1000 gm*	*Rate in infants 1000-1499 gm*	*Rate in infants 1500-1999 gm*	*Rate in infants 2000-2499 gm*
Dowding and Barry[13]	20.2	46.7	15.7	3.2
Meberg[16]		13*		14*
Hagberg[11]		90*	30	6
Jarvis et al.[9]		3.5*		7.4*
Pharoah et al.[17]		44*		9.7*
Stanley and Watson[14]		26.9*	17.2	8

*Includes the lower-birth-weight group.

Table V. Percentages of serious mental retardation (IQ <50) in cases of cerebral palsy by birth weight*

Study	*Age (yr)*	*Country*	*Rate in all weights (%)*	*Rate of serious mental retardation in <2500 gm with cerebral palsy (%)*	*Rate of serious mental retardation in >2499 gm with cerebral palsy (%)*
Nelson and Broman[21]	7	United States†	26	15	33
Hagberg et al.[11]	4	Sweden	14	13	16
Pharoah et al.[18]	5 (mean)	England	36	27	40
Composite rate			25	18	30

*Includes infants or children who died (cumulative incidence).
†Collaborative Perinatal Project.

Table VI. Percentage of cerebral palsy in those with serious mental retardation (IQ <50)*

Study	*Age (yr)*	*Country or state*	*With cerebral palsy in serious mental retardation population (%)*
McQueen et al.[23]	7-10	Canada	15.4
Jacobsen and Janicki[24]	<21	New York	15.8
Gustavson et al.[25]	5-16	Sweden	18.6
McDonald[26]	10	Quebec	12.8
Oregon State Board of Health[27]	<20	Oregon	15.8
Drillien et al.[28]†	7.5-14.5	Scotland	22.9
Composite rate			16.8

*Surviving at time of study.
†IQ <55.

studies where births did occur solely in the 1980s were the Meberg study[16] and the Pharoah et al.[17] studies. Some studies, published quite recently, were based on births from as far back as the 1960s or earlier, before the use of neonatal intensive care and before the means were established to prevent erythroblastosis fetalis.

With the advent of neonatal intensive care units in the 1970s, the survival of very-low-birth-weight infants has improved. The average survival rate beyond the neonatal period for the <1500-gm infant in developed countries was 15% to 40% in the early 1970s and 60% to 75% in the mid-1980s.[31] Although there is concern that, with this increased survival, the incidence of cerebral palsy among LBW infants has increased, we found the rate to be fairly stable at about 15 per 1000 LBW births (<2500 gm) since the 1950s (Fig. 1). For the very-low-birth-weight infant (<1500 gm) this rate is higher. Studies found rates of cerebral palsy of 13 to 90 per 1000 for neonatal survivors of 500 to 1500 gm at birth (Table IV). Other studies have had findings of a stable rate of neurologic impairment along with an increased survival rate for very-low-birth-weight babies. A review by Hagberg et al.[32] found that where a country had a higher perinatal mortality rate to begin with and a greater drop in perinatal mortality rate with the advent of improved neonatal care, the rate of cerebral palsy did not increase noticeably. However, when the rate was lower to begin with and neonatal care has been used with increasingly smaller babies, the cerebral palsy rate has increased to a greater extent. The implication is that babies who have needed minimal to moderate improved neonatal care to survive tend to have low morbidity, whereas the much smaller babies who need intensive, long-term care to survive are more likely to have permanent neurologic problems. Hagberg et al.[32] sees a 40 times higher live-birth prevalence of cerebral palsy in the very-low-birth-weight infant than in the normal-birth-weight infant.

One problem we encountered was the cutoff point for LBW babies. Some studies included the 2500 gm infant in the LBW group and others did not. This discrepancy could significantly change the final figure. Another problem was the interchangeable use of the terms LBW and preterm.[22] With these problems, the figures on cerebral palsy by birth weight are a close approximation of cerebral palsy by birth weight. An average

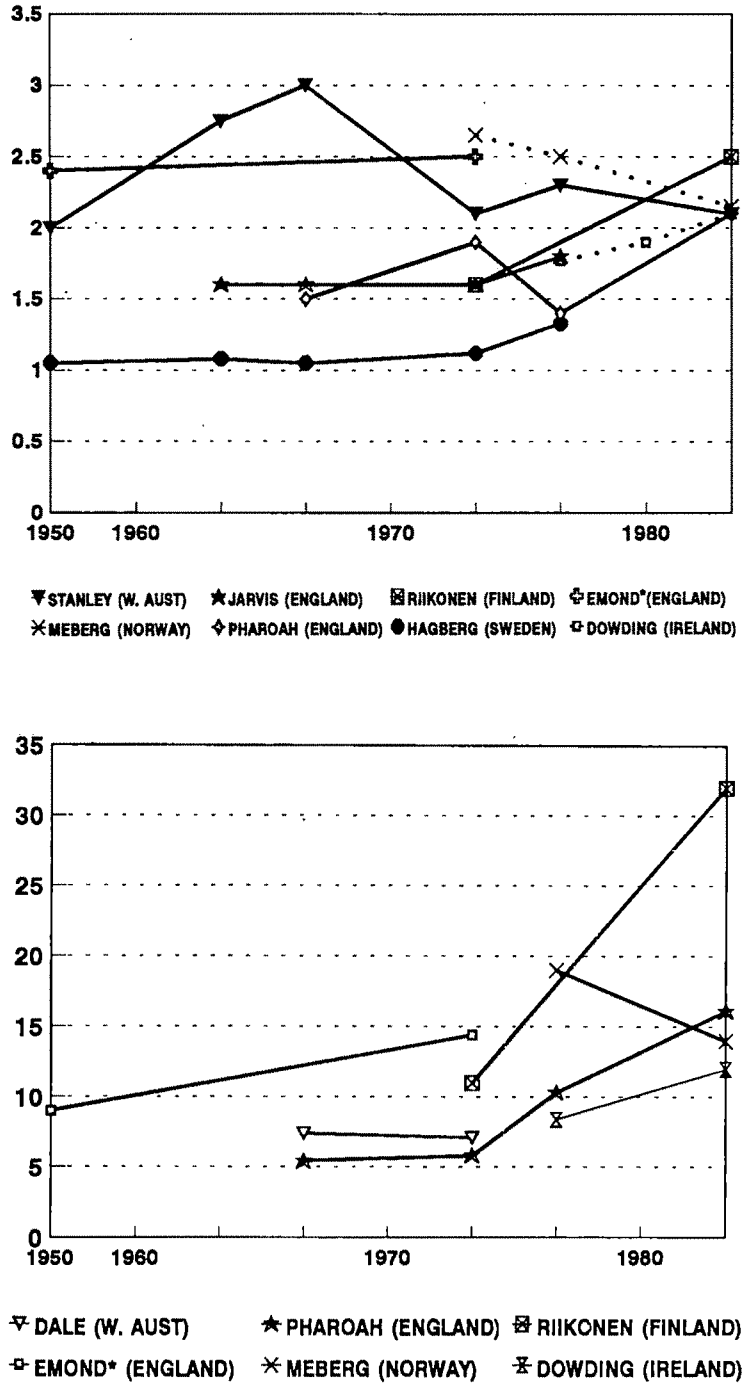


Fig. 1. Rates of cerebral palsy for all birth weights per 1000 live births *(top)* and per 1000 live births <2500 gm *(bottom)*. *Asterisk,* Neonatal survivors.

of 36% of all cerebral palsy occurred in the LBW population. Therefore approximately 5.3% of the birth population contributed 36% of the cases of cerebral palsy. When studies providing cerebral palsy rates over periods of time are considered, the rate for infants weighing >2499 gm was stable at 1 to 1.5 per 1000 births of infants of that birth weight. Our composite figure, which includes more studies, was 1.8.

There appeared to be some consistency in the rates of cerebral palsy for the different birth weights, so one would think that overall rates could be tentatively extrapolated on the basis of a combination of the LBW rate, which is higher in the United States and has remained relatively unchanged at about 6.8%,[33] and the survival of these infants. Because the percentage of LBW infants varies from country to country, we would then expect the percentage of cerebral palsy occurring ing FHR β-sympathetic and parasympathetic tone, re-

in different birth weights to vary in a similar fashion. However, this was not the case. Other variables seemed to be involved. Besides the socioeconomic and ethnic compositions of the population that appear to be associated with LBW, one also needs to consider health policies in and within different countries concerning access to prenatal care and the resuscitation and treatment of very small newborns.

As early as 1897, Freud noted that there appeared to be different types of cerebral palsy that probably had different origins, so they should be studied separately. Others have reiterated this message more recently.[18] Birth weights and types of cerebral palsy seem to be intertwined so it is difficult to discuss one without discussing the other. Diplegia, which occurs more often among infants of LBW, was 34% of all cerebral palsy cases, whereas LBW infants were 36% of all cases of cerebral palsy (although these two do not completely overlap).

The rates of serious mental retardation (IQ <50) occurring along with cerebral palsy also differed by birth weight. The composite rate of serious mental retardation in those with cerebral palsy was 25% for all birth weights, 18% for LBW, and 30% for those weighing >2499 gm. Therefore 75% of all children with cerebral palsy have IQs >50 with most of those having IQs >70, and the occurrence of serious mental retardation is more common with the normal-birth-weight infants who have cerebral palsy. Among all infants with serious mental retardation, the rate of cerebral palsy was approximately 16.8%. However, the sources of the populations for this last figure need to be considered. Many of these studies were based on institutionalized populations. Also, because these studies were based on survivors, it underestimates the overall incidence.

Thus we may estimate that intrapartum cause for cerebral palsy is <0.5 per 1000 term births and that active prevention for those infants awaits better understanding of cause of the disease, because the usual monitoring methods in labor do not allow for that diagnosis.[1] It is more probable that understanding and preventing premature birth and better means of antepartum surveillance are more likely to contribute to the prevention of cerebral palsy.

To the obstetrician the incidence of cerebal palsy has major clinical meaning. It is clear that the target population for cerebral palsy still hovers at about 2.7 per 1000 live births at 4 to 10 years of age. About 36% of cerebral palsy will have origins in infants weighing <2500 gm, with increasing risk for cerebral palsy with each decrement in infant birth weight. The emphasis in research should be on finding causes of and solutions for early prenatal problems that contribute to births at very low weights, to births of small-for-gestational-age infants, and to births of otherwise prenatally damaged infants. Tort reform needs to acknowledge these unknowns in the etiology of cerebral palsy, so the clinician can be enabled to practice his or her best without having to do so defensively.

In the term infant the incidence of cerebral palsy will be about 1.8 per 1000 of children with that birth weight. Thus the target for more than 90% of all deliveries is small. It is also important to understand that past estimates of origins of cerebral palsy for term infants are divided into perinatal (25% to 30%) causes and the remainder probably prenatal or unknown causes. A portion of those with perinatal causes will be intrapartum causes.

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Home uterine activity monitoring in France: A randomized, controlled trial

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OBJECTIVES: We assessed the effectiveness of ambulatory tocodynamometry in reducing the preterm delivery rate in women at risk of preterm delivery such as women with risk factors and women previously hospitalized and discharged.

STUDY DESIGN: In four public maternity units these women were randomly allocated to two groups: 84 had home uterine activity monitoring and daily midwife contact and 84 were given the standard care for high-risk women, which generally includes home visits by community midwives.

RESULTS: The proportion of deliveries before 37 weeks' gestation was slightly higher in the monitored group than in the control group (32% vs 22%). The corresponding odds ratio was 1.7 (95% confidence interval: 0.9 to 3.5).

CONCLUSION: Although the sample was small, these results suggest that home uterine activity monitoring was probably not beneficial to the population studied, or at least that any benefit would have been too small to justify extending this monitoring in this high-risk population. (Am J Obstet Gynecol 1992;167:424-9.)

Key words: Term Guard system, preterm delivery, uterine activity, prenatal screening, prevention

The identification of women at increased risk of preterm labor is one of the major issues in preventing preterm deliveries. At present, screening of threatened preterm labor is mainly conducted on the basis of the findings of vaginal examinations and the perception of contractions by women, but these criteria have limited usefulness. Certain signs of cervical maturation, especially the dilatation of the internal os, are predictive of preterm delivery[1-4] but have low sensitivity.[5] Furthermore, self-reported contractions are poorly correlated to uterine activity[6] and to preterm delivery.[3, 4]

Another approach to enhancing early diagnosis of threatened preterm labor was recently developed on the basis of assessment of uterine activity by ambulatory tocodynamometer in high-risk women. It was observed that women who subsequently experienced preterm labor had greater uterine activity than women who labored at term, and this difference was found up to several weeks before the onset of labor.[7] In the United States, the effectiveness of home uterine activity monitoring and daily nursing contact has been assessed in several randomized, controlled trials in which this type of care was found to reduce the preterm delivery rate[8] or to permit earlier diagnosis of preterm labor.[9-11]

However, because home uterine activity monitoring has positive effects in the United States does not guarantee that it will be effective in other settings. In France an active policy is pursued during pregnancy to prevent preterm delivery by routine vaginal examinations, bed rest, admission to the hospital, prescription of β-sympathomimetics and home visits by community midwives. To ascertain whether under these conditions home uterine activity monitoring reduces the number of preterm deliveries, we conducted a randomized, con-

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Table I. Demographic and social characteristics of women

	Monitored group	*Control group*
Maternal age (yrs, mean ± SD)	28.6 ± 4.7	28.3 ± 4.8
	$n = 80$	$n = 83$
Number of previous pregnancies (%)		
0	31	27
1	27	30
≥2	42	43
	$n = 84$	$n = 83$
Not living with the child's father* (%)	7	5
	$n = 83$	$n = 75$
Low social class† (%)	35	42
	$n = 76$	$n = 71$

*Any marital status.

†Father was either manual worker or had no occupation. Mothers not cohabiting were not considered in this variable.

trolled trial in which a group of women using the Term Guard uterine activity monitor (Tokos Medical Corporation, Santa Ana, Calif.) was compared with a control group given the usual obstetric care provided high-risk women in France.

Population and method

The trial included two groups of women: (1) women who had been discharged and sent home after hospitalization for threatened preterm labor (discharged group), and (2) women at high risk for preterm delivery (high-risk group). In the latter group, the risk factors were twin gestation, previous delivery at <35 completed weeks of gestation or cervical cerclage during the current pregnancy. Women were enrolled between 24 and 34 weeks of pregnancy.

We compared two groups of women: a monitored group and a control group. The monitored group was instructed at home in the use of the ambulatory tocodynamometer. The device was used for 1 hour every morning and evening. A midwife at the Tokos Medical Center contacted the women every day by telephone. The women were asked to state their symptoms and transmit the data recorded concerning their uterine activity. The women were also visited once a week by a Tokos Center midwife. Women with persistent symptoms or contractions in excess of their established baseline contraction frequency were sent to the outpatient clinic or the inpatient ward.

The women in the control group had home visits by a community midwife once or twice a week; this was the standard care prescribed by the participating centers for high-risk women. In addition, the women in both the monitored and control groups were given prenatal care by obstetricians or midwives at the outpatient clinics and were hospitalized if necessary.

Because the objective of the trial was to assess the Term Guard system in current medical practice, we did not alter this practice in the participating centers, and no specific recommendations were made to physicians regarding the diagnosis of threatened preterm labor or tocolytic treatments. In France women with severe symptoms of threatened preterm labor are usually admitted to the hospital and are administered intravenous β-sympathomimetics. The other tocolytic treatments are mainly inhibitors of prostaglandin synthesis and progesterone.

Our study was carried out in the four public maternity units located in Lyon. The women were enrolled between October 1988 and May 1989. After obtaining their informed consent we allocated them to the monitored and control groups by randomization with sealed envelopes. In each maternity unit randomization was done separately within the discharged and high-risk groups.

In all, 168 women were randomized into the monitored or control groups in a ratio of 1:1. As regards the two populations included in this trial, the overall sample comprised 74 women who had been discharged from the hospital (37 monitored and 37 control group women), and 94 high-risk women (47 monitored and 47 control). The risk factors of the latter group were the following: twin gestation (55%), previous delivery at <35 weeks (24%) and cervical cerclage (21%). One woman in the control group was lost to follow-up after enrollment, so that the analysis was conducted on 167 women: 84 in the monitored group and 83 in the control group.

During the study data were collected at entry and after delivery. At entry an information sheet was completed before randomization to confirm the eligibility of each participant and assess the risk of preterm delivery from the state of the cervix and the contractions perceived by the woman. After delivery data on prenatal care, delivery, and pregnancy outcome were obtained from hospital case notes.

Gestational age was assessed on the basis of last menstrual period and ultrasonography. If there were dis-

Table II. Risk factors at study entry

	Monitored group	*Control group*
Previous adverse outcome* (%)	19 $n = 84$	33 $n = 81$
Twin gestation (%)	30 $n = 84$	32 $n = 83$
Treated threatened preterm labor† (%)	49 $n = 83$	36 $n = 83$
Perceived contractions at study entry (%)	51 $n = 82$	43 $n = 76$
Cervix at study entry (%)		
Dilatation of the internal os ≥1 cm	16 $n = 82$	14 $n = 77$
Length ≤1 cm	21 $n = 82$	24 $n = 78$

Differences are not significant except previous adverse outcome ($p < 0.05$).
*Stillbirth, neonatal death, or delivery at <37 full weeks of gestation.
†Treatment before study entry with β-sympathomimetics or inhibitors of prostaglandin synthesis.

agreement the ultrasonographic data were used. Preterm delivery (before 37 full weeks' gestational age) was the primary outcome criterion. However, participating physicians or midwives sometimes stopped tocolytic treatment or uterine monitoring at 35 weeks, and this cut-off point was also selected for the analysis. To distinguish threatend preterm labor from preterm delivery, threatened preterm labor was defined as preterm labor not followed by delivery within 24 hours after the onset of the first symptoms (contractions, dilatation of the internal os of the cervix, or premature rupture of the membranes).

Statistical analysis was done by the χ^2 test and Student's t test. Analysis was stratified by group (discharged or high-risk). Results for gestational age and birth weight were given as odds ratios, and the corresponding confidence intervals were calculated according to the method of Mantel-Haenszel.

Results

At study entry the monitored and control groups were comparable for maternal age, number of previous pregnancies, family status, and social class (Table I). We found that the proportion of women who had a history of stillbirth, neonatal death, or delivery before 37 weeks of gestation was smaller in the monitored group than in the control group (Table II). No difference between the two groups was observed for two other risk factors: the proportion of twin gestations and episodes of threatened preterm labor treated by β-sympathomimetics or inhibitors of prostaglandin synthesis before study entry. The two groups were also comparable with regard to the state of the cervix and the contractions perceived by the women at study entry. Furthermore, the women in both groups were enrolled at a similar gestational age: 28.9 weeks (±3.3) in the monitored group and 28.6 weeks (±2.9) in the control group.

In the monitored group one woman refused to participate in the monitoring during the initial contact by a Tokos Center midwife; later, two others failed to comply with the Term Guard regimen and the monitoring was stopped. These three women were kept in the monitored group for the analysis. The average length of monitoring was 42.6 days (±42.0). During this period the women had 3.3 home visits (±1.7) by a Tokos Center midwife. For 69% of the women the monitoring was definitely stopped because the length of gestation was close to 37 weeks. The two other main reasons for the cessation of monitoring were admission to hospital for preterm labor or delivery (21%) or diagnosis of another complication (6%).

In the control group 87% of the women had home visits by community midwives (Table III). The average length of this care system was 42.3 days (±47.0). The women in both the monitored and control groups had three visits to the outpatient clinic between study entry and delivery.

The proportion of women admitted to the hospital for threatened preterm labor or other pregnancy complications was 37% in the monitored group and 40% in the control group. In the monitored group 21 (25%) women were admitted for threatened preterm labor. Of these, three had cervical dilatation >1 cm and two had premature rupture of membranes. In the control group, of the 16 (19%) women admitted for threatened preterm labor, two had cervical dilation >1 cm and four had premature rupture of membranes. In both groups all the women admitted for threatened preterm labor were treated with tocolytic agents; nevertheless, intravenous β-sympathomimetics were prescribed for 13 (15%) women in the monitored group and 6 (7%) in the control group. Overall prescription of contraction inhibitors (intravenous or oral β-sympathomimetics, indomethacin, or progesterone) was more frequent in the monitored group than in the control group (79% vs 59%; $p = 0.006$).

Table III. Prenatal care after study entry

	Monitored group	*Control group*	*p Value*
Type of care (%)			
Term Guard system	99	0	
Home visiting system	0	87	$p < 0.001$
Neither	1	13	
	$n = 84$	$n = 83$	
Visits to the outpatient clinic (mean ± SD)	3.0 ± 2.3	3.0 ± 1.9	NS
	$n = 75$	$n = 77$	
Admission to hospital* (%)	37	40	NS
	$n = 84$	$n = 83$	
Admission for threatened preterm labor* (%)	25	19	NS
	$n = 84$	$n = 83$	
Intravenous β-sympathomimetics* (%)	15	7	NS
	$n = 84$	$n = 83$	
Treatment for inhibition of contractions† (%)	79	59	$p = 0.006$
	$n = 81$	$n = 78$	

NS, Not significant.

*Not followed by delivery within 24 hours.

†β-sympathomimetics, indomethacin, or progesterone, prescribed in the inpatient ward or at the outpatient clinic.

In the monitored group six babies were stillborn or died before discharge (Table IV). Four were preterm (between 25 and 28 weeks). In the control group there were three stillbirths or deaths, one of which was preterm (25 weeks). The proportion of deliveries at <37 weeks of gestation was 32% in the monitored group and 22% in the control group (odds ratio = 1.7; 0.9 to 3.5). The trend was similar in the two stratified groups, that is, in the discharged group (22% vs 13%) and the high-risk group (40% vs 28%). The proportions of deliveries before 35 weeks were 19% and 11%, respectively, in the monitored and control groups (odds ratio = 1.9; 0.8 to 4.7). Among all the singletons and twins delivered, the proportion of babies weighing <2500 gm was similar in the two groups (36 vs 34%, odds ratio = 1.1; 0.6 to 2.1).

Comment

In our study we did not find home uterine activity monitoring to significantly reduce the number of preterm deliveries. Our sample size was not large enough to enable us to reach a definite conclusion regarding the effect of the Term Guard system. Nevertheless, our results showed no trend that argued in favor of this type of care and, if this system had a positive effect, it would be a very small one. According to the range of the odds ratio for preterm delivery, the relative decrease in the preterm delivery rate is very unlikely to be >10% through home uterine activity monitoring. Thus there is poor evidence that the Term Guard system can be useful in our population because the beneficial effect that could be observed would be too small to justify extending the use of such a system.

We originally planned to include 900 women in the trial. This sample size was based on the expected preterm delivery rates, which we estimated to be 25% for the control group on the basis of previous French studies.[12, 13] Thus a trial including 900 women had a 95% chance of showing a significant difference at the 5% level if the preterm delivery was reduced from 25% in the control group to 15% in the monitored group (two-tailed test). It was not possible to reach this sample size because the Tokos Medical Corporation was no longer funded in France. However, according to the preterm delivery rates observed in our study, the sample size originally planned would have been too small to show a significant difference between the monitored group and the control group.

Our results cannot be explained by a higher risk of preterm delivery in the monitored group than in the control group, because the two groups were similar as regards all risk factors except for a history of adverse pregnancy outcome, which was more frequent in the control group. A few more women were treated for threatened preterm labor before enrollment in the monitored than in the control group, but at study entry dilatation of the internal os of the cervix was equally frequent in both groups. After study entry we found that treatments for the inhibition of contractions, administration of intravenous β-sympathomimetics and hospitalization for threatened preterm labor were slightly more frequent in the monitored group than in the control group. This could have been because of the presence of more women with threatened preterm labor in the monitored group than in the control group. It could also be the very consequence of uterine activity monitoring because the aim of such monitoring is to detect contractions that would otherwise be unknown. Because the doctors knew the results of the monitoring, they might have diagnosed a larger number of cases of threatened preterm labor in the monitored group than in the control.

Table IV. Pregnancy outcome

	Monitored group	*Control group*	*Odds ratio**	*95% CI*
Stillbirth	1/109	1/110		
Death before discharge	5/109	2/110		
Weeks' gestation (%)				
≤32	7	3		
33-34	12	8		
35-36	13	11		
≥37	68	78		
	(84 women)	(83 women)		
Gestational age <37 wks (%)	32	22	1.7	0.9-3.5
	(84 women)	(83 women)		
Birth weight <2500 gm (%)	36	34	1.1	0.6-2.1
	(107 newborns)	(110 newborns)		

CI, Confidence interval.
*Monitored versus control.

During the trial attention was paid to the adequate use of home uterine activity monitoring and nursing support. This care system was provided by the French branch of the Tokos Medical Corporation. The monitoring device used, the organization of home care, and the standards applied to the interpretation of the data obtained by monitoring were similar to those prevailing in the United States. The midwife in charge of the Tokos Center was experienced in the prevention of preterm births and had been trained in several Tokos Centers in the United States. This care system was tested in two different settings in France before the trial began.[14, 15] Furthermore, in the trial protocol home visits by a Tokos midwife were added to the care usually provided by Tokos Centers to make sure that the monitored group would not be at a disadvantage compared with the control group.

One of the published randomized controlled trials that assessed Term Guard effectiveness showed clear evidence of a decrease in the preterm delivery rate in the overall group of women who used a uterine activity monitor compared with those who used uterine self-palpation (15% vs 45%).[8] However, the results were based on a small sample (34 and 33 women, respectively), and the confidence interval of the odds ratio for preterm delivery was large. In other randomized controlled trials in which women monitored at home were compared with women given standard care and education on self-palpation for uterine contractions,[9-11] the positive effects observed on the preterm delivery rate were restricted to the women who experienced preterm labor after study entry, and no overall conclusion was reached for the entire group included in these trials.

It is often suggested that the Term Guard system might be effective in preventing preterm births because uterine activity monitoring twice a day and frequent contacts with nurses would help to diagnose preterm labor at an earlier stage, when the state of the cervix is considered to be favorable for tocolysis. This possibility has been confirmed by several randomized controlled trials in which, at the first preterm labor episode, women who were monitored daily had less advanced cervical dilatation than those given standard care, and there were fewer tocolysis failures in the former group than in the latter.[8-11] We were unable to verify this hypothesis ourselves because we only knew the state of the cervix for women who had threatened preterm labor and who were delivered >24 hours after admission to hospital. However, among these women we found no trend towards earlier diagnosis of threatened preterm labor in the monitored group.

If the Term Guard system has a beneficial effect in reducing the preterm delivery rate, the question is raised as to whether it is the monitoring data or the frequent nurse contacts that result in improved outcome. The results of several randomized controlled trials suggest that the information supplied by monitoring might be less effective than the support provided by daily contacts with a nurse. Thus Iams et al.[16-17] showed that the preterm delivery rate was not significantly different in the group monitored by the Term Guard system and in a group of women who only had daily contact with a Tokos Center nurse. Dyson et al.[18] compared a monitored group with a group of women who used the monitor and also had this daily contact but for whom the monitoring data were not available for care management. Dyson et al. found no significant difference in preterm delivery rates between the two groups. However, in patients with twin gestation they observed a significant reduction in the numbers of very low birth weight babies and admissions to the intensive care nursery in the group for whom the monitoring data were used. Porto et al.[19] made a similar comparison among women who had either single or multiple gestation and found no difference in the frequency of preterm delivery between the monitored group and the group with blind data. Nurses might, therefore, have a major role in the Term Guard system by providing

social support and paying attention to all the preterm labor symptoms reported by the women.[20]

If this is the case our finding that home uterine activity monitoring failed to reduce the number of preterm deliveries might be the result of the great attention devoted to the screening of threatened preterm labor and the efforts made to prevent preterm deliveries in France. For example, books aimed at pregnant women emphasize the risk of preterm delivery and the measures that can be taken to prevent it.[21-23] These publications also describe in detail the symptoms of preterm labor, thus inciting women to contact their physician and comply with the medical decisions. Furthermore, French doctors have a very active approach to the prevention of preterm deliveries. The effectiveness of all the measures taken to avoid this pregnancy outcome is not always known and some of these measures, such as home visits by community midwives,[24,25] have not been observed to have positive effects on the preterm delivery rate. Nevertheless, the overall policy conducted to prevent preterm deliveries may have beneficial results. The introduction of a new type of care such as the Term Guard system may not necessarily be useful within this framework, although it might be effective in a different medical environment.

Further research is needed to test whether home uterine activity monitoring improves the prevention of preterm birth as it was recently expressed by a multidisciplinary workshop.[26] Thus we will know in which countries or populations beneficial effects may be expected.

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Maternal hyperoxygenation in the treatment of intrauterine growth retardation

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OBJECTIVE: In the current study the efficacy of maternal hyperoxygenation on growth-retarded fetuses was evaluated.

STUDY DESIGN: Thirty-six pregnant women with intrauterine growth retardation were studied. The patients were divided in oxygen-treated (n = 17) and untreated (n = 19) groups. Doppler analysis of the fetal circulation was performed on the arrival to the hospital, after 12 hours, and thereafter on alternate days until delivery. Fetal blood was sampled by cordocentesis for immediate blood gas analysis at entrance to the study and the day of delivery.

RESULTS: Significant improvement in Doppler flow patterns in treated patients were found when compared with untreated women. The Doppler variations were associated with complementary modifications in fetal blood gas. These differences resulted in a significant modification in perinatal mortality with an incidence of 29% and 68% ($p < 0.01$) in treated and untreated groups, respectively.

CONCLUSION: Our data suggest a benefit of maternal hyperoxygenation in the treatment of fetal growth retardation. (AM J OBSTET GYNECOL 1992;167:430-5.)

Key words: Hyperoxygenation, growth retardation, Doppler, cordocentesis

Uteroplacental insufficiency leads to intrauterine growth retardation (IUGR), which is a major cause of perinatal mortality and postnatal morbidity.[1, 2] IUGR and oligohydramnios in the second and early third trimesters of pregnancy indicate a poor prognosis for the fetus. These conditions may complicate about 1% of pregnancies.[3]

Arterial Po_2 in the fetus is 25% to 33% of the arterial Po_2 in the adult. Some authors referred to this as "physiologic hypoxia" even though it represents a normal state of fetal oxygenation.[4] However, in spite of the low Po_2, fetal blood is capable of transporting large amounts of oxygen from the placenta to the fetus. This is possible because (1) the hemoglobin of fetal red blood cells has a high affinity for oxygen and (2) the rate of perfusion of fetal organs is high in comparison with oxygen requirements.

Fetal hypoxia is induced by any decrease in fetal oxygenation below the normal level. Such a decrease may induce a reduction in the Po_2 of umbilical venous blood (hypoxic hypoxia). Chronic intrauterine hypoxia, hypercapnia, acidosis, hyperlacticemia, and erythroblastosis occur in many growth-retarded fetuses.[5]

Current management includes early diagnosis, bed rest, and antenatal monitoring of fetal well-being until the fetus reaches a stage of viability that allows delivery. In the current prospective, randomized study the value of long-term maternal hyperoxygenation in comparison with the traditional management of bed rest was evaluated in the treatment of growth-retarded fetuses.

Patients and methods

From October 1989 through August 1991 we studied 36 pregnant women at 26 to 34 weeks' gestation with IUGR. All the patients gave their informed consent to participate in the study. Patients included in the study fulfilled the following criteria: known last menstrual period, regular menses, absence of maternal diabetes, fetal ultrasonographic examination before 22 weeks with biometric data consistent with duration of amenorrhea, singleton fetus, no fetal malformation or chromosomal defects; reduced amount of amniotic fluid (oligohydramnios was defined by the absence of any amniotic fluid pocket measuring ≥2 cm in a vertical axis), abnormal Doppler flow velocity waveforms in maternal-fetal circulation (±2 SD of our reference ranges), ultrasonographic measurements of fetal abdominal circumference <5th percentile of our reference ranges, and reactive nonstress test.

The patients admitted in the study were randomly assigned to two different treatments: (1) the traditional

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Table I. Demographic and clinical data in oxygen-treated and untreated patients (mean ± SE)

	Treated (n = 17)			*Untreated (n = 19)*			*Significance between groups*	
	Admission	*Delivery*	*Significance (p)*	*Admission*	*Delivery*	*Significance (p)*	*Admission (p)*	*Delivery (p)*
Maternal age (yr)	28.1 (25-35)			29.4 (29-36)			NS	
Gestational age (wk)	30.2 ± 0.5	31.7 ± 0.3		29.7 ± 1.6	30.9 ± 0.1		NS	NS
Parity								
0	8 (47%)			10 (53%)			NS	
1	8 (47%)			7 (37%)			NS	
>1	1 (6%)			2 (10%)			NS	
Time from diagnosis to parturition (days)	10.1 ± 3.5			9 ± 3.1			NS	
Estriol (ng/ml)	41.4 ± 7	58.1 ± 8.4	NS	39.1 ± 6.3	40.3 ± 0.8	NS	NS	NS
Human placental lactogen (μg/ml)	3.4 ± 0.2	7.3 ± 0.6	NS	2.9 ± 0.2	2.9 ± 0.6	NS	NS	NS
Hematocrit (%)	37.8 ± 1.3	37.5 ± 1.7	NS	38.4 ± 1.5	38.6 ± 1	NS	NS	NS
Uric acid (mg/dl)	4.8 ± 0.2	5.1 ± 0.1	NS	4.9 ± 0.1	5.5 ± 0.8	NS	NS	NS
Mean platelet volume (fl)	12.1 ± 0.3	13.3 ± 0.5	NS	11.4 ± 0.2	12.2 ± 0.5	NS	NS	NS
Platelets (1000/ml)	165 ± 11	131 ± 10	NS	189 ± 17	152 ± 8	NS	NS	NS
Oligohydramnios	17 (100%)			19 (100%)			NS	

NS, Not significant.

management of bed rest (plus antihypertensive medication, when necessary) and (2) the same management plus humidified 55% oxygen at a rate of 8 L/min administered continuously (except during meals, etc.) through an MC face mask for 24 hours a day.[3]

Ultrasonographic assessment of amniotic fluid volume was performed on alternate days until parturition. A weekly ultrasonographic measurement of fetal abdominal circumference was performed. Both assessments were performed with the patients in a semirecumbent position with a 3.5 MHz convex array real-time scanner (Ansaldo AU 940, Genova, Italy).

Doppler analysis of maternal-fetal circulation was performed on the arrival of patients to the hospital, after 12 hours (only on umbilical artery), and thereafter on alternate days until parturition. Flow velocity waveforms were recorded with a duplex pulsed Doppler (Ansaldo AU 940) with a 3.5 MHz convex transducer. Low-frequency Doppler signals originating from vessel wall motion were eliminated by a 100 Hz low-frequency filter. No fetal breathing or intervening fetal motion activity occurred during recording. Several arteries were analyzed transabdominally. We calculated the resistance index (Resistance index = Systolic − Diastolic/Systolic)[6] for the uterine and umbilical artery and the pulsatility index (Pulsatility index = Systolic − Diastolic/Mean)[7] for the descending thoracic aorta and middle cerebral artery. The time-averaged mean velocity in the descending aorta was calculated[9] (the pulsed sample volume was placed in the fetal descending aorta just above the level of diaphragm; the angle of insonation was between 50 and 60 degrees; and the results express the mean velocities over the cardiac cycle of the maximum frequencies recorded). For each examination we obtained the mean value of three consecutive waveforms.

Fetal blood was sampled by cordocentesis[10, 11] for immediate blood-gas analysis at entrance to the study while the mother was breathing air and again on the day of delivery while the mother was breathing oxygen. Cordocentesis was performed by an aseptic technique without maternal fasting, sedation, and local anesthesia. The umbilical cord vessel sampled was identified by ultrasonography as either vein or artery by turbulence produced after injection of 200 μl of saline solution.[10, 11] The fetal origin of blood was subsequently confirmed by the Kleihauer test. Fetal blood (100 μl) was collected into heparinized syringes, and blood-gas analysis was performed with a Radiometer AB-330 blood gas anlayzer (Radiometer, Copenhagen). On the day of delivery, after a further informed consent, cordocentesis was repeated, indicating to the patients that gas analysis would be useful to understand about fetal well-being.

The Doppler and cordocentesis indices were not revealed to the labor staff but were correlated with pregnancy outcome.

During the study all patients had the following maternal-fetal functional indices measured every 3 days: human placental lactogen, estriol, hematocrit, platelet count, mean platelet volume, and uric acid.

The potential harm of hyperoxygenation for the mother's lung was studied by serial determinations of pulmonary vital capacity.[12]

Statistical analysis. An unpaired *t* test was used to calculate the significance of the difference among the

Table II. Fetal transabdominal Doppler velocimetry in oxygen-treated and untreated patients (mean ± SE)

	Treated (N = 17)						
	Admission		*After 12 hours*		*Delivery*		*Significance (p)*
Resistance index							
Uterine artery		0.78 ± 0.06				0.78 ± 0.08	NS
Umbilical artery	*N* = 11	EDF negative	*N* = 11	EDF negative	*N* = 10	EDF negative	NC
	N = 2	RF	*N* = 2	RF	*N* = 1	RF	NC
	N = 4	0.84 ± 0.04	*N* = 4	0.83 ± 0.01	*N* = 6	0.78 ± 0.06	NS
Pulsatility index							
Thoracic aorta		5.8 ± 0.4				5.0 ± 0.1	<0.05
Middle cerebral artery		2.4 ± 0.2				3.1 ± 0.1	<0.05
Time-averaged mean velocity (cm/sec)							
Thoracic aorta		16 ± 2				20.7 ± 1	<0.05

EDF, End-diastolic flow; *RF*, reverse flow; *NS*, not significant; *NC*, not calculated. Note: In the treated group in one case the reverse flow changed in end-diastolic flow negative and in two cases the end-diastolic flow negative changed with the appearance of diastolic flow. In the untreated group in three cases the diastolic flow end-diastolic flow disappeared and in two cases the end-diastolic flow negative changed in reverse flow.

Table III. Cordocentesis indices* in oxygen-treated and untreated patients (mean ± SE)

	Treatment (N = 17)		
	Admission normal range	*Delivery normal range*	*Significance (p)*
P_{O_2} (mm Hg)	21.2 ± 2.4 (24-50)	27.7 ± 3.3 (23-48)	<0.01
P_{CO_2} (mm Hg)	43.7 ± 1.8 (31-42)	38.0 ± 0.6 (32-43)	<0.05
pH	7.31 ± 0.03 (7.36-7.45)	7.34 ± 0.01 (7.37-7.45)	<0.05
Oxygen saturation (%)	57.0 ± 2.8 (60-89)	71.2 ± 3.4 (68-88)	<0.01

NS, Not significant.

*All the sample were obtained from umbilical vein.

groups. A value of $p < 0.05$ was considered statistically significant.

Results

The 36 women were divided into oxygen-treated ($n = 17$) and untreated ($n = 19$) patients. In the treated group the duration of oxygen therapy was 10.1 ± 3.4 (mean ± SE) days.

At admission to the hospital the fetal abdominal circumference was as indicated in Fig. 1.

Many indices, such as demographic and clinical data, were not significantly different between the treated and untreated groups (Table I). Except for uterine artery waveforms, significant improvements were found in Doppler flow patterns (Table II) in treated patients compared with untreated women. The intraobserver variations were 5.8%. The Doppler variations were associated with complementary modifications in fetal blood gas (Table III). These differences resulted in a significant modification in perinatal mortality with an incidence of 29% and 68% ($p < 0.01$) in treated and untreated groups, respectively. A single case of intrauterine death resulted in the untreated group. The indications for delivery and perinatal outcome were similar in both groups (Table IV). In all the cases the birth weight was below the fifth percentile of Italian standards. In the treated patients the surviving fetuses had little morbidity with no severe neonatal complications. In the untreated group one of the surviving fetuses developed intraventricular hemorrage with subsequent severe handicaps. The fetal growth velocity was not significantly affected by therapy.

No fetal complications, except eight cases of transient bradycardia, were registered as a consequence of fetal blood sampling in either group.

No significant variations in maternal pulmonary capacity were found in the treated patients. The adverse effects of high oxygen concentrations on the fetus were not evaluated in the postnatal period because in our cases the rise in fetal blood P_{O_2} resulted in values within the normal range.

The obstetric management was decided by the labor staff on the interpretation of fetal heart rate tracings or the deterioration of maternal conditions.

Untreated (N = 19)				Significance between groups	
Admission	After 12 hours	Delivery	Significance (p)	Admission	Delivery
0.81 ± 0.04		0.84 ± 0.08	NS	NS	NS
N = 12 EDF negative	N = 12 EDF negative	N = 13 EDF negative	NC	NC	NC
N = 1 RF	N = 1 RF	N = 3 RF	NC	NC	NC
N = 6 0.86 ± 0.03	N = 6 0.83 ± 0.02	N = 3 0.86 ± 0.04	NS	NS	NS
5.8 ± 0.8		6.0 ± 0.3	NS	NS	<0.01
2.7 ± 0.1		1.9 ± 0.1	<0.01	NS	<0.01
17.1 ± 2		16.0 ± 0.4	NS	NS	<0.05

Untreated (N = 19)			Significance between groups	
Admission (normal range)	Delivery (normal range)	Significance (p)	Admission (p)	Delivery (p)
23.7 ± 0.9 (24-50)	20.0 ± 1.0 (23-48)	<0.05	NS	<0.01
39.1 ± 2.7 (31-42)	45.0 ± 1.5 (32-43)	<0.05	NS	<0.01
7.31 ± 0.05 (7.36-7.45)	7.30 ± 0.02 (7.37-7.45)	NS	NS	<0.01
58.3 ± 2.4 (60-89)	50.9 ± 4.1 (68-88)	<0.05	NS	<0.01

Table IV. Delivery data and perinatal outcome in oxygen-treated and untreated patients (mean ± SE)

	Treated (n = 17)	Untreated (n = 19)	Significance (p)
Indication for delivery			
Preeclampsia	3 (18%)	5 (26%)	NS
Intrauterine death	—	1 (5%)	NS
Biophysical profile	14 (82%)	13 (69%)	NS
Vaginal delivery	—	1 (5%)	NS
Cesarean delivery	17 (100%)	18 (95%)	NS
Male fetuses	14 (73%)	14 (74%)	NS
Female fetuses	3 (27%)	5 (26%)	NS
Birth weight (gm)	1070 ± 139	997 ± 134	NS
Birth weight <1000 gm	8/17 (47%)	12/19 (63%)	NC
Perinatal death	5 (29%)	12 (63%)	
	(29%)	(68%)	<0.01
Stillbirth	—	1 (5%)	
Severe neonatal complication	—	1 (5%)*	NS

NS, Not significant; *NC*, not calculated.
*Intraventricular hemorrhage.

Comment

Starting from the hypothesis that the most likely cause of IUGR is an impaired uteroplacental or fetal-placental perfusion with consequent reduced maternal-fetal exchange, maternal oxygen therapy was suggested to improve fetal metabolic conditions.

In the current study the efficacy of maternal hyperoxygenation was rather evident. After 12 hours of ther-

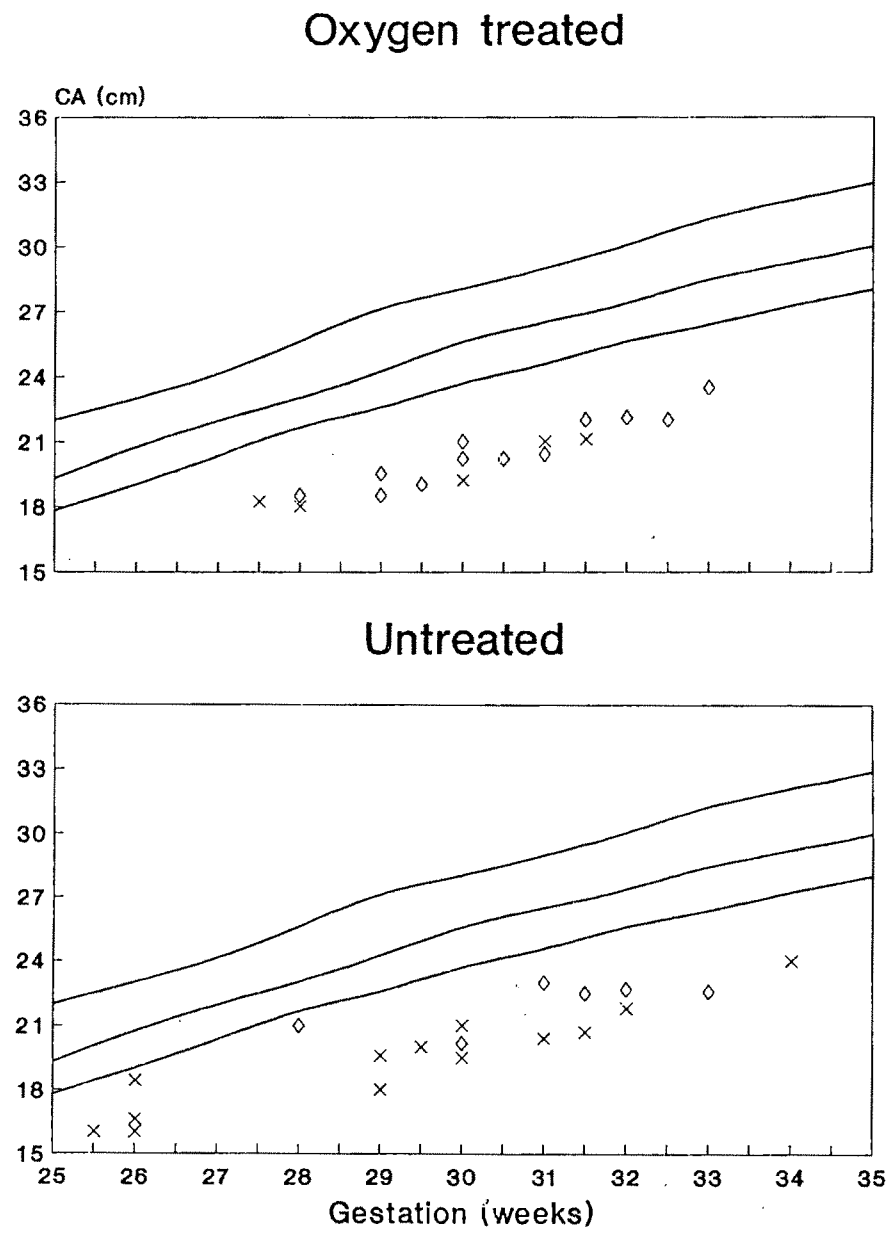


Fig. 1. Fetal abdominal circumference *(CA)* at admission to hospital in 17 IUGR fetuses who were subsequently treated with maternal hyperoxygenation, compared with 19 untreated fetuses. There were 18 (◇) surviving fetuses and 18 (×) dead fetuses.

apy no evident changes of Doppler flow waveforms were recorded. However, on the day of delivery, after a mean period of 10 days of oxygen therapy, modifications of the Doppler patterns were shown. At the level of umbilical artery in one case the reverse flow was modified to end-diastolic flow negative, and in two cases of end-diastolic flow negative a diastolic flow reappeared a few days before the delivery. In the treated patients a significant improvement of Doppler flow velocity waveforms in other districts was observed. Such variations were associated with a progressive improvement in blood-gas indices. On the other hand, untreated patients showed a worsening of the "brain-sparing effect." Hypoxia, hypercapnia, and acidosis worsened in the untreated group.

As previously described, some IUGR fetuses are hypoxemic, hypercapnic, hyperlacticemic, and acidotic.[5] Several mechanisms in the production of hyperlacticemia have been suggested. Increased placental production, impaired fetal gluconeogenesis, and inadequate oxygen supply represent possible sites of lactate hyperproduction.[13-15] In the current study the evaluation of maternal and fetal blood lactate concentration was not done.

The maternal hyperoxygenation in hypoxic and severely growth-retarded fetuses improved and maintained fetoplacental oxygenation. This effect resulted independently from the immediate response of the fetal-placental vasculature. Furthermore, the maternal hyperoxygenation modifying the fetal blood-gas concentrations and consequently the chemoreceptor-mediated vascular reactivity reversed the brain-sparing effect, probably restored an aerobic metabolism, and resulted in the decreased incidence of fetal death and neonatal complications. This is an indirect confirmation of what occurs in animal experiments, in which the more depressed the Po_2 and pH are, the more probable the development of brain injury is and the more difficult resuscitation is.[4]

In conclusion, maternal oxygen therapy improved the fetal condition and survival but was inadequate in increasing the placental transfer of nutrients, because fetal growth velocity resulted unaffected.[3]

The study suggests a benefit of oxygen therapy for the treatment of severe IUGR and should encourage further studies.

We thank Dr. Felice Petraglia for his kind collaboration in preparing the manuscript.

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Behavioral treatment of menopausal hot flushes: Evaluation by ambulatory monitoring

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OBJECTIVES: We attempted to develop an effective behavioral treatment for menopausal hot flushes and to determine the active behavioral components of this procedure.
STUDY DESIGN: Thirty-three women with frequent menopausal hot flushes were randomly assigned to receive eight sessions of training in paced respiration, muscle relaxation, or α-wave electroencephalographic biofeedback (placebo control). Hot flushes were objectively measured by means of 24-hour ambulatory monitoring of the sternal skin conductance level.
RESULTS: Subjects undergoing paced respiration had significant reductions in hot flush frequency and respiration rate, as well as increases in tidal volume.
CONCLUSION: Paced respiration training may be a useful treatment alternative for the reduction of hot flushes in women who cannot tolerate hormone replacement therapy. (AM J OBSTET GYNECOL 1992;167:436-9.)

Key words: Hot flush, menopause, relaxation, sympathetic nervous system

Hot flushes are the most common symptom of the climacteric and occur in the vast majority of menopausal women. Although hormone replacement therapy is an effective treatment for hot flushes, it may be contraindicated for some women who have an increased risk of cancer. Central sympathetic activation is increased in women with hot flushes,[1-3] and agents such as clonidine, which inhibit this activation, have been shown to ameliorate hot flushes in some studies.[4-6]

Behavioral relaxation methods have been shown to reduce sympathetic activity in normal subjects[7-9] and in some clinical groups.[10] In a pilot study[11] we showed that a combination of progressive muscle relaxation exercises and slow, deep breathing reduced the incidence of self-reported hot flushes by about 50%. Since two treatment procedures were combined in that study, it was not possible to determine which component was responsible for the therapeutic effect. Therefore in the current investigation, the muscle relaxation and paced respiration exercises were administered to two separate groups. α-Wave electroencephalographic biofeedback was chosen as the placebo control procedure because it elicits subjective reports of relaxation without its physiologic effects.[12] We also sought to objectively determine the effects of the treatments with the use of 24-hour ambulatory monitoring of the sternal skin conductance level. This measure has been shown to correlate highly with patients' reports of hot flushes.[13, 14]

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Material and methods

Thirty-three postmenopausal women were recruited from newspaper advertisements requesting women for participation in a research study of behavioral treatments for hot flushes. All women reported having at least five hot flushes per day and had been amenorrheic for at least 1 year. Three women had previously taken estrogen but stopped at least 1 year before the current study. All women gave written informed consent according to procedures approved by our institutional review board.

The women were randomly assigned, in equal numbers, to one of three behavioral treatment groups. Each woman received eight 1-hour, biweekly treatment sessions. Patients undergoing paced respiration had abdominal and thoracic chest excursions recorded with strain gauges and a polygraph. A research assistant viewed the polygraph tracings and instructed the patient to breathe at six to eight cycles/min and to increase the amplitude of the abdominal tracing. Patients learning muscle relaxation were given live training by a research assistant using previously published methods.[15] Patients were initially trained to systematically tense and then relax 16 gross muscle groups and to distinguish between sensations of tension and relaxation. In later sessions the procedure was reduced to seven and then to four muscle groups. α-Wave feedback patients received visual feedback for the production of 8 to 13

Table I. Demographic characteristics of patients (means ± SD)

	Treatment group		
	Paced respiration	*Muscle relaxation*	*α-Wave feedback*
Age (yr)	50.7 ± 8.6	53.1 ± 6.0	52.9 ± 5.7
Weight (lb)	141.3 ± 37.1	146.3 ± 17.9	145.7 ± 14.0
Years postmenopausal	5.0 ± 5.3	7.0 ± 6.7	6.3 ± 5.3
Menopause			
Natural	9	9	9
Surgical	2	2	2
Race			
White	6	8	7
Black	5	3	4

Hz electroencephalographic activity according to previously published methods.[11] In the procedure the patient watches a light that is activated when 8 to 13 Hz electroencephalographic activity reaches a preset amplitude level. The training is done in three 10-minute trials separated by 5-minute rest periods.

Before treatment, all women received 24-hour ambulatory monitoring of the sternal skin conductance level to detect hot flushes.[13,14] Two Meditrace disposable silver–silver chloride electrodes, 1.5 cm in diameter, were filled with 0.05 mol/L potassium chloride paste and attached over the sternum. Sternal skin conductance level signals were recorded on an Oxford Medilog 9000 recorder with a constant voltage circuit. The Medilog tapes were replayed at 60× real time with an Oxford Medilog playback unit, an analog-to-digital converter, and an IBM computer. The computer was programmed to detect any sternal skin conductance level increase ≥2 μmho in 30 seconds, which served as the objective definition of a hot flush. We have previously demonstrated[14] that the concordance between this measure and patient reports was 95% for spontaneous hot flushes recorded in the laboratory and 86% for those recorded during ambulatory monitoring. The ambulatory recording was repeated after completion of the last treatment session.

Before treatment, each patient also received a test of her ability to voluntarily relax. After a 10-minute rest period, the patient was instructed to relax as deeply as possible for 5 minutes, followed by a second 10-minute rest period. During the entire session, the patient's respiration rate and tidal volume were recorded with a Sensormedics MMC metabolic cart. This procedure was repeated after the last treatment session.

Pretreatment group differences in age, weight, years since menopause, hot flush frequency, and respiratory measures were tested with one-way analyses of variance. Hot flush frequency data were next analyzed with a two-way (Group × Pretreatment/Posttreatment) repeated-measures analysis of variance. The significant interaction effect was then decomposed into the pretreatment/posttreatment simple effects for each group.[16] Respiratory measures were analyzed with three-way (Group × Period × Pretreatment/Posttreatment) repeated-measures analyses of variance. As described, significant interactions were broken down into lower-order simple effects for further analysis. Possible relationships between changes in hot flush frequency and demographic variables were tested with Pearson product-moment correlation coefficients (age, weight, years since menopause) and *t* tests (race, type of menopause). The minimum level of statistical significance was $p < 0.05$.

Table II. Hot flush frequency during 24-hour ambulatory monitoring (means ± SD)

	Treatment group		
	Paced respiration	*Muscle relaxation*	*α-Wave feedback*
Pretreatment	15.7 ± 8.1	14.2 ± 9.8	10.3 ± 7.3
Posttreatment	9.6 ± 6.2*	13.6 ± 10.6	12.0 ± 10.2

*$p < 0.02$, significantly different from pretreatment value.

Table III. Respiration measurements during voluntary relaxation tests (means ± SD)

	Treatment group		
	Paced respiration	*Muscle relaxation*	*α-Wave feedback*
Pretreatment			
Rate (cycles/min)	13.7 ± 2.2	12.5 ± 2.7	12.8 ± 2.2
Tidal volume (L/breath)	0.42 ± 0.07	0.50 ± 0.14	0.53 ± 0.10
Posttreatment			
Rate (cycles/min)	10.2 ± 3.3*	11.5 ± 2.7	13.7 ± 3.2
Tidal volume (L/breath)	0.55 ± 0.27†	0.48 ± 0.16	0.47 ± 0.11

*$p < 0.015$, significantly different from corresponding pretreatment value.

†$p < 0.03$, significantly different from corresponding pretreatment value.

Results

Before treatment, there were no significant differences among the three groups in age, weight, or time since menopause (Table I) or in the frequency of sternal skin conductance level–defined hot flushes recorded during ambulatory monitoring (Table II). The analysis of hot flush frequency demonstrated a significant ($p < 0.05$) Group × Pretreatment/Posttreatment interaction effect. Simple effects tests showed that hot flush frequency decreased significantly for the paced

respiration group ($p < 0.02$) but not for the muscle relaxation ($p > 0.2$) or α-wave feedback ($p > 0.5$) groups.

There were no group differences in respiration rate or tidal volume during the pretreatment voluntary relaxation test (Table III). The analysis of variance on respiration rate showed a significant ($p < 0.02$) Group × Pretreatment/Posttreatment interaction effect. Simple effects analyses showed a significant Pretreatment/Posttreatment decline on this measure for the paced respiration group ($p < 0.015$) but not for the other two groups (both $p > 0.4$). For tidal volume a significant ($p < 0.05$) Group × Pretreatment/Posttreatment interaction effect was also found. Simple effects tests showed a significant pretreatment/posttreatment increase in tidal volume for the paced respiration group ($p < 0.03$) but not for the muscle relaxation ($p > 0.8$) or α-wave feedback ($p > 0.5$) groups.

There were no significant relationships between any pretreatment variable and the pretreatment/posttreatment change in hot flush frequency.

Comment

In the current study patients who received paced respiration training significantly reduced their frequency of hot flushes, increased their tidal volume, and slowed their respiration rates. Patients who received muscle relaxation exercises or α-wave feedback showed no significant changes in hot flush frequency or in either respiration measure. Taken together with our previous findings,[11] these results show that training in slow, deep breathing alone will result in a significant reduction in the occurrence of menopausal hot flushes.

This effect was documented by 24-hour monitoring of an objective measure of hot flushes and therefore is not subject to the limitations of self-report data. Moreover, our recordings were performed over a 24-hour period; therefore they accurately detected both sleeping and waking hot flushes. Self-reports of events occurring during sleep are obviously less reliable than objective physiologic data, particularly since not all hot flushes result in awakening.[17]

It has previously been shown that yohimbine, an α_2-adrenergic antagonist, provokes hot flushes[2] and that clonidine, an α_2-adrenergic agonist, ameliorates them.[2, 4-6] Yohimbine increases central norepinephrine release by blocking inhibitory, presynaptic α_2-adrenergic receptors[18] and clonidine acts in the opposite manner.[19] The main metabolite of brain norepinephrine is 3-methoxy-4-hydroxyphenyl glycol,[20] and we have shown that plasma levels of this metabolite are significantly elevated in menopausal women with hot flushes as compared with levels in those without them.[3] Taken together, these data suggest that elevated central sympathetic activation is involved in the initiation of menopausal hot flushes.

We hypothesize that the slow, deep breathing exercises act in some way to modulate this activation and thereby reduce the frequency of hot flushes. However, the connection between changes in respiration and central sympathetic activation remains to be made. In cross-sectional studies, Schaefer[21] compared slow, deep breathers having high alveolar carbon dioxide levels with fast, shallow breathers having low carbon dioxide levels. The former group had slower resting heart rates, reduced adrenal cortical activity, and decreased autonomic responsiveness compared with the latter group, but measures of central sympathetic activity were not obtained. Further research in this area would be of great interest.

In conclusion, we have shown that training in a simple breathing procedure results in a significant reduction in menopausal hot flushes as measured over a 24-hour period. This technique may be useful for women with hot flushes who are unable to receive hormone replacement therapy.

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Increased lipolytic activity and high ratio of free fatty acids to albumin in sera from women with preeclampsia leads to triglyceride accumulation in cultured endothelial cells

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OBJECTIVE: The null hypothesis of this study was that the triglyceride accumulation in endothelial cells exposed to sera from preeclamptic women was determined by the presence of triglyceride-rich lipoproteins in the sera.
STUDY DESIGN: The accumulation of triglycerides in cultured endothelial cells was studied using incorporation of tritiated glycerol.
RESULTS: Triglyceride-rich lipoproteins in the patient sera contributed little to the endothelial triglyceride accumulation. However, sera from preeclamptic women were found to have a higher molar ratio of free fatty acids to albumin compared with sera from women with normal pregnancies (1.6 ± 0.5 vs 0.9 ± 0.4, respectively, $p < 0.025$). In addition, sera from preeclamptic women, compared with sera from normal pregnancies, showed enhanced lipolytic activity (release of free fatty acids 0.85 ± 0.29 vs 0.17 ± 0.16 mmol/ml per 24 hours, respectively: $p < 0.025$) that further increased the free fatty acids/albumin ratio.
CONCLUSION: Sera from preeclamptic women have both a higher ratio of free fatty acids to albumin and increased lipolytic activity, resulting in enhanced endothelial uptake of free fatty acids, which are further esterified into triglycerides. (Am J Obstet Gynecol 1992;167:440-7.)

Key words: Preeclampsia, endothelium, hyperlipidemia, free fatty acids, lipolysis

Preeclampsia is a pregnancy-specific disorder characterized by increased blood pressure, urinary loss of proteins, edema, and activation of the hemostatic system.[1] It has become evident that many of these pathophysiologic changes may be explained by alterations in the maternal vascular endothelium.[2] However, factors that can cause disturbance of the endothelial cell functions in preeclampsia have not been identified.

Pregnant women have altered lipid metabolism as reflected in a marked hyperlipidemia.[3] Among those in whom preeclampsia develops, an additional change in the lipid metabolism seems to be present because the plasma levels of triglycerides are further increased as compared with those of women with normal pregnancies.[3] From the area of cardiovascular research there is increasing evidence that hyperlipidemia can cause endothelial dysfunction.[4] We have therefore raised the question whether the endothelial dysfunction of preeclampsia is causatively related to the alterations in the metabolism of lipids.

Recently we reported that sera from women with preeclampsia induced accumulation of triglycerides in endothelial cells.[5] This accumulation was accompanied by altered endothelial cell function as demonstrated by reduced prostacyclin release. In the current work we have studied the mechanism(s) responsible for the triglyceride accumulation in the endothelial cells. We demonstrate that both an increased ratio of free fatty acids to albumin and an enhanced lipolytic activity of sera from women with preeclampsia result in higher endothelial uptake of free fatty acids, which are further esterified into triglycerides.

Material and methods

Chemicals. Albumin, triglycerides, insulin, transferrin, oleic acid, stearic acid, and linoleic acid were all obtained from Sigma (St. Louis). Ham's F-10 medium and penicillin-streptomycin solution were purchased from Gibco (Grand Island, N.Y.). Tissue culture equipment was supplied by Costar (Cambridge) and Falcon (New Jersey, England). 1(3)^{3}H-glycerol, 99.9 GBq/mmol, were purchased from Amersham (Arlington Heights, Ill.). All organic solutions were of reagent grade.

Patient selection. A previously healthy normotensive

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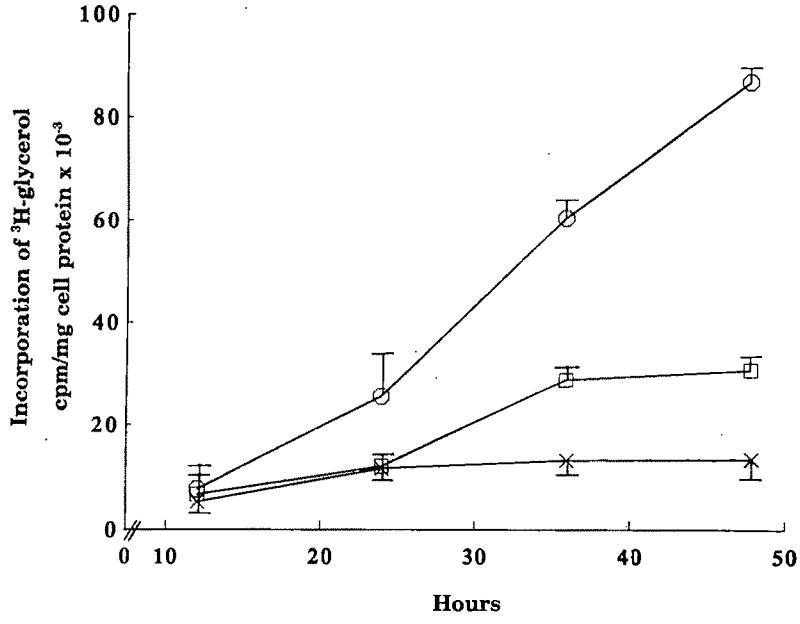


Fig. 1. Effect of various times of exposure of endothelial cells to sera from preeclamptic women (○), control pregnancies (□), and nonpregnant women (×) on incorporation of tritiated (^3H) glycerol into endothelial triglycerides. Each *point* represents mean ± SD of triplicate incubations.

woman was considered to have preeclampsia if the blood pressure after 20 weeks of gestation was >140/90 mm Hg on at least two occasions 6 hours apart. In addition, proteinuria of >300 mg/24 hours was required for the diagnosis. The control group consisted of healthy women of similar gestational age and parity with uncomplicated pregnancies and nonpregnant women.

Blood samples. Informed consent was obtained from each women. Blood samples were drawn after 8 to 10 hours of fasting. The blood was allowed to coagulate for 90 to 120 minutes at room temperature. Sera were obtained by centrifugation at 800*g* for 10 minutes and immediately placed at −70° C. Care was taken to handle the sera from preeclamptic and control women similarly and in parallel.

Cell cultures. Endothelial cells were isolated from the veins of umbilical cords obtained from normal pregnancies of women not included in the control group.[5] The cells were seeded in 2 cm² wells in 1 ml of culture medium consisting of Ham's F-10 medium supplemented with 2.5% (vol/vol) bovine calf serum, 7.5% (vol/vol) horse serum, penicillin (100 IU/ml), streptomycin (100 μg/ml), and amphotericin (2.5 μg/ml). Primary cultures were obtained from a new umbilical cord for each experiment, and the cells were used 2 to 3 days after seeding.

Preparation of isolated lipoprotein fractions. Very-low-density lipoprotein was prepared from freshly obtained sera from preeclamptic and control women by ultracentrifugation at density 1.006 gm/ml at 100,000*g* under sterile conditions for 24 hours at 4° C.[6] The fractions obtained were used immediately after preparation.

Table I. Endothelial content of triglycerides and tritiated glycerol incorporation into triglycerides after 60 hours' exposure to sera from preeclamptic women or sera from normal pregnancies

Sera	*μg Triglycerides per mg cell protein*	*Counts/min/mg × 10^{-3}*
Preeclamptic women	117 ± 13	140 ± 12
Normal pregnancies	29 ± 4	11 ± 2

Values are mean ± SD of triplicate incubations.

Measurement of tritiated glycerol incorporation into cellular triglycerides. In the experiments where tritiated glycerol incorporation into endothelial triglycerides was measured, the procedure was as follows: 5 μCi/ml tritiated glycerol and 0.1 μmol/ml unlabeled glycerol (both final concentrations) were added 12 hours before the end of each incubation (unless otherwise indicated) in a volume of 50 μl per well (containing

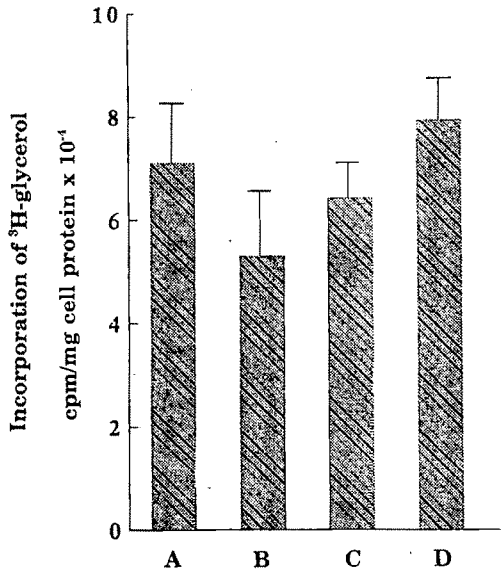


Fig. 2. Incorporation of tritiated glycerol into endothelial triglycerides in presence or absence of very-low-density lipoprotein. Endothelial cells were exposed for 36 hours to unfractionated preeclamptic serum *(A)*, very-low-density lipoprotein–deficient serum *(B)*, or very-low-density lipoprotein–deficient serum reconstituted with very-low-density lipoprotein (*C*, 1 μmol triglycerides per milliliter; *D*, 2.5 μmol triglycerides per milliliter). Each *bar* represents mean ± SD of triplicate incubations.

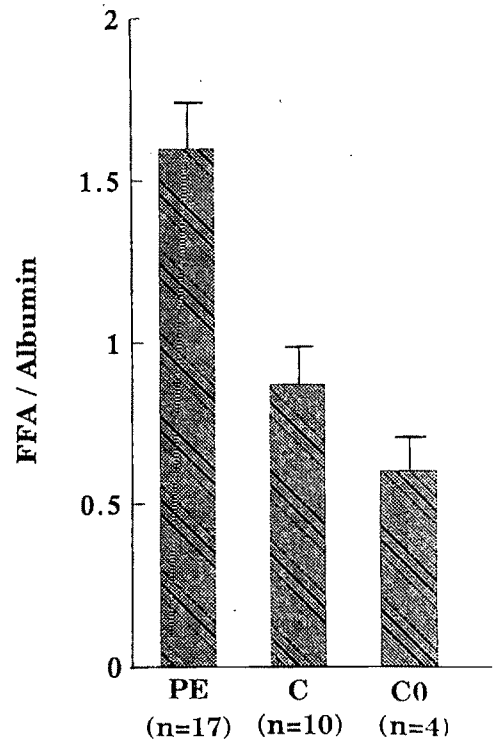


Fig. 3. Molar ratio of free fatty acids *(FFA)* to albumin in sera from women with preeclampsia *(PE)*, women with uncomplicated pregnancies *(C)*, and nonpregnant women *(C0)*. Duplicate determinations were done for each individual. Numbers of individual sera and SEM are indicated for each *bar*. Difference between sera from preeclamptic and normally pregnant women was significant, $p < 0.025$.

1 ml of medium). To end an experiment the cells were washed four times with phosphate-buffered saline solution with Hepes,[5] and cellular lipids were extracted in situ by exposure to 1 ml of hexane per well for 2 hours. The lipids were then separated by thin-layer chromatography on precoated silica gel plates (Polygram, Machery-Nagel, Germany) in a solvent system of petroleumether-diethyl ether acetic acid (85:15:1). The triglyceride-containing spots were scraped off, and the radioactivity was measured (Rackbeta 1217, LKB, Pharmacia, Oslo). Preliminary experiments showed that 85% of the hexane extractable lipids were found in the triglyceride fraction. In some of the experiments the endothelial content of triglycerides was determined as described previously.[5] Cellular protein was determined according to the method of Lowry et al., as described by Henriksen et al.[6]

Lipid staining. For staining of lipids the procedure was as follows: The cells were washed twice in Hepes and phosphate-buffered saline solution, fixed in 50% ethanol, stained in sudan red for 2 to 3 minutes, quickly washed in 50% ethanol, followed by another washing in purified water. The cells were afterstained in hematoxylin for 2 to 3 minutes, washed in purified water, and finally exposed to saturated lithium carbonate for a few seconds.

Experimental protocol

Effect of time of exposure of endothelial cells to sera from preeclamptic and normally pregnant women and nonpregnant women on incorporation of tritiated glycerol into cellular triglycerides. The cells were washed twice[5] with Ham's F-10 medium and then incubated for 12, 24, 36, and 48 hours with 1 ml of Ham's F-10 medium containing 30% (vol/vol) sera from either preeclamptic, normally pregnant women or nonpregnant women. Tritiated glycerol incorporation into endothelial triglycerides was measured as described above.

Cell incubations in presence and absence of very-low-density lipoprotein. Freshly isolated very-low-density lipoprotein in serum from a preeclamptic woman was added back to the very-low-density lipoprotein–poor bottom in two different concentrations (1.0 or 2.5 μmol of triglyceride per milliliter). The cells were washed twice[5] and then incubated with either unfractionated serum or the very-low-density lipoprotein–poor bottom with or without the two different concentrations of very-low-density lipoprotein. Tritiated glycerol incorporation into endothelial triglycerides was

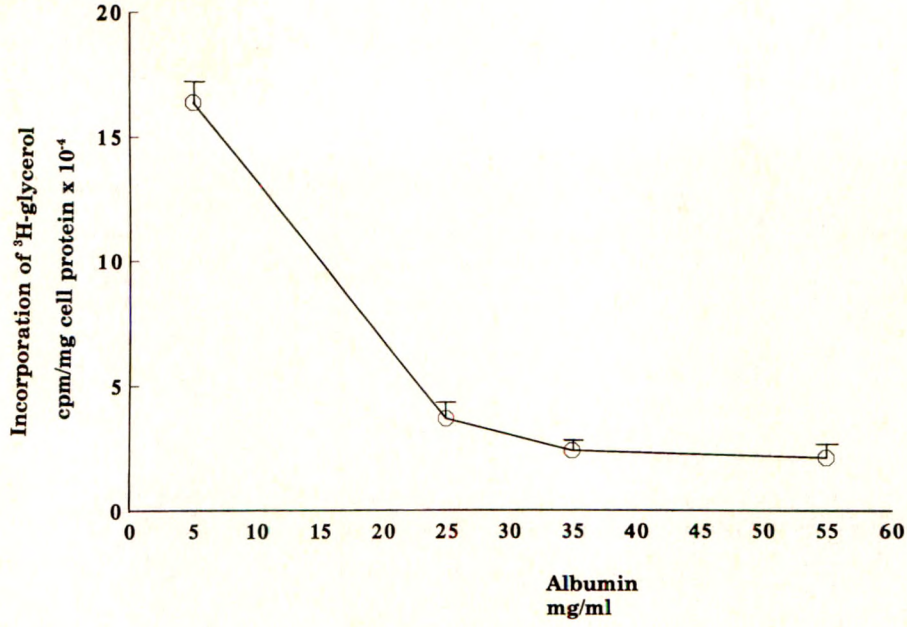


Fig. 4. Effect of albumin on incorporation of tritiated glycerol into endothelial triglycerides. Endothelial cells were exposed for 48 hours to serum from women with preeclampsia containing increasing amounts of albumin. Final albumin concentrations in culture media are given on *abscissa*. Each *point* represents mean ± SD of triplicate incubations.

measured as described in the preceding text. Parallel cultures were stained for lipids.

Cell incubations with various ratios of free fatty acids/albumin

Heat-inactivated sera. Three sera from women with preeclampsia and three sera from normally pregnant women (freshly obtained and stored at −70° C until start of experiment) with different ratios of free fatty acids/albumin were heat inactivated at 57° C for 60 minutes. Then the cells were washed twice and incubated with Ham's F-10 medium containing 30% (vol/vol) of the heat-treated sera. After 2 days the medium was renewed, followed by another 2 days' incubation. Tritiated glycerol incorporation into cellular triglycerides was measured as described.

Addition of albumin to serum from a woman with preeclampsia at start of cell incubations. Increasing amounts of albumin was added to serum from a woman with preeclampsia with an original albumin concentration of 17 mg/ml. The endothelial cells were then washed twice and exposed to Ham's F-10 medium containing 30% (vol/vol) of the serum with or without the additional albumin (final concentrations in the culture media were 5, 25, 35, and 55 mg/ml). After 48 hours' incubation tritiated glycerol was added, and after another 24 hours' incubation incorporation into endothelial triglycerides was measured as described. Parallel cultures were stained for lipids.

Addition of free fatty acids to sera from normally pregnant women at start of cell incubations. A total of 2 μmol of free fatty acids (360 μg of oleic acid, 120 μg of stearic acid, 90 μg of linoleic acid, and 30 μg of arachidonic acid) was dissolved in heptane and evaporated under nitrogen. The dry material was dissolved in 0.5 ml of ethanol containing 2.2 μmol of sodium hydroxide. After evaporation under nitrogen the free fatty acid salts were dissolved in 100 μl of purified water at 60° C. The free fatty acids were added to 2 ml of serum from normally pregnant women in 10 μl aliquots with gentle shaking at 37° C. The endothelial cells were then exposed to Ham's F-10 medium containing 30% (vol/vol) of the serum from normally pregnant women with or without the additional free fatty acids. The final total concentrations of free fatty acids in the culture medium were 0.74, 0.86, 1.11, and 1.61 μmol/ml. After 24 hours' incubation tritiated glycerol was added, and after another 24 hours' incubation the incorporation into triglycerides was measured as described in the preceding text.

Lipolytic activity in sera from preeclamptic and normally pregnant women and nonpregnant women. The lipolytic activity was determined as the difference in the concentration of free fatty acids at the start and end of incubations at 37° C for the times indicated in the figure legends. In three experiments the sera were heat inactivated by incubation for 60 minutes at 57° C. The concentration of free fatty acids in the sera or the culture media was measured with a kit for determi-

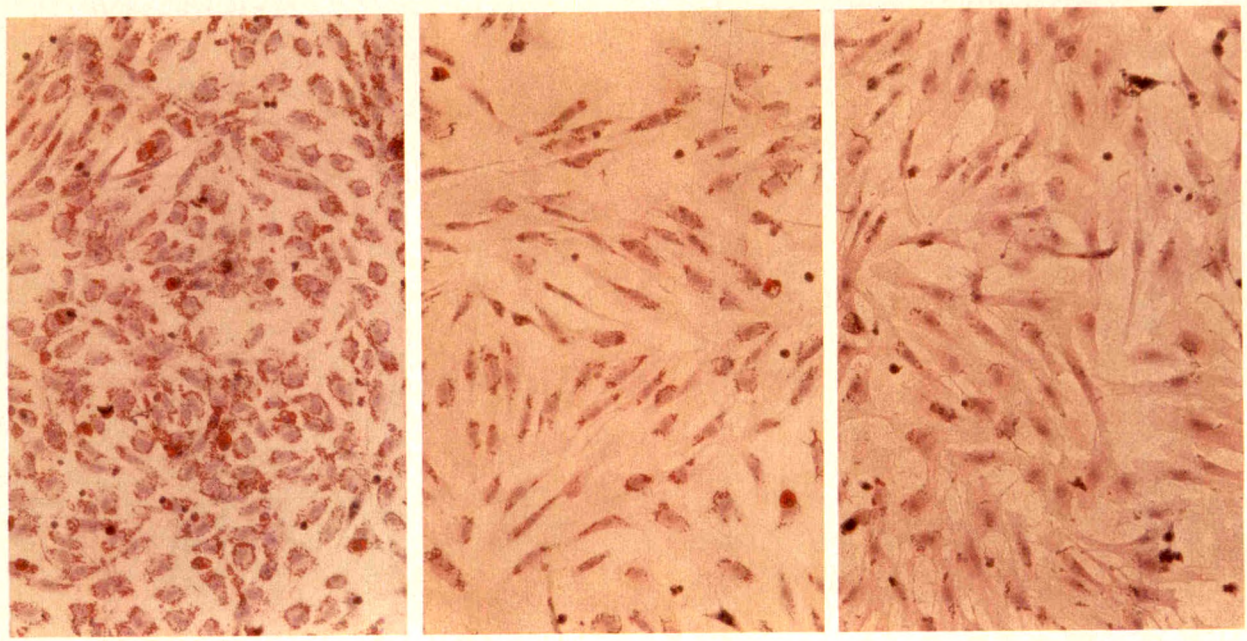


Fig. 5. Content of sudanophilic granules in endothelial cells exposed to preeclamptic serum *(left panel)* or to preeclamptic serum with additional albumin, 8 mg/ml *(middle panel)* or 18 mg/ml *(right panel)*.

nation of nonesterified fatty acid kit (Wako, Germany). Initial experiments showed that the release of free fatty acids during clotting of the blood contributed to <8% of the free fatty acid content of the sera. Furthermore, when sera were tested against plasma, similar levels of free fatty acids were found.

Statistical analysis. The Student *t* test was used.

Results

In five experiments with sera from five different individuals the incorporation of tritiated glycerol into endothelial triglycerides was up to 20 times higher in endothelial cells exposed to sera from women with preeclampsia than in the controls. Table I shows an experiment in which the tritiated glycerol incorporation was twelve times higher than in the control. The endothelial content of triglycerides was increased four times compared with that in the controls. The incorporation of tritiated glycerol was close to linear during a 48-hour incubation with serum from women with preeclampsia (Fig. 1).

Very-low-density lipoprotein is the major carrier of serum triglycerides and can lead to triglyceride accumulation in cells after endocytic uptake.[7] Addition of very-low-density lipoprotein from sera from women with preeclampsia to albumin-containing Ham's F-10 medium did not lead to accumulation of sudanophilic granules in the endothelial cytoplasm (data not shown). Furthermore, removal of very-low-density lipoprotein decreased the tritiated glycerol incorporation into triglycerides by about 20% compared with that observed for unfractionated sera from women with preeclampsia ($p > 0.025$). When very-low-density lipoprotein was added back to very-low-density lipoprotein–poor sera from women with preeclampsia, a 20% increase in the tritiated glycerol incorporation was found (Fig. 2).

A second possible cause of triglyceride accumulation in cells is increased uptake of exogenous free fatty acids.[8] The major determinant of the rate of cellular uptake of free fatty acids is the ratio of free fatty acids to albumin.[9] As shown in Fig. 3, sera from women with preeclampsia had a significantly higher free fatty acids/albumin ratio compared with sera from normally pregnant and nonpregnant women ($p < 0.025$). Given that the triglyceride accumulation in endothelial cells exposed to sera from women with preeclampsia is due to enhanced free fatty acid uptake, addition of albumin should have an inhibitory effect. As shown in Fig. 4, addition of increasing amounts of albumin to sera from women with preeclampsia almost completely inhibited the incorporation of tritiated glycerol into triglycerides. Furthermore, the cellular lipid accumulation induced by the sera from women with preeclampsia could be reversed by addition of albumin (Fig. 5). Conversely, addition of free fatty acids to a sera from normally pregnant women resulted in increased incorporation of tritiated glycerol into triglycerides (Fig. 6). A cor-

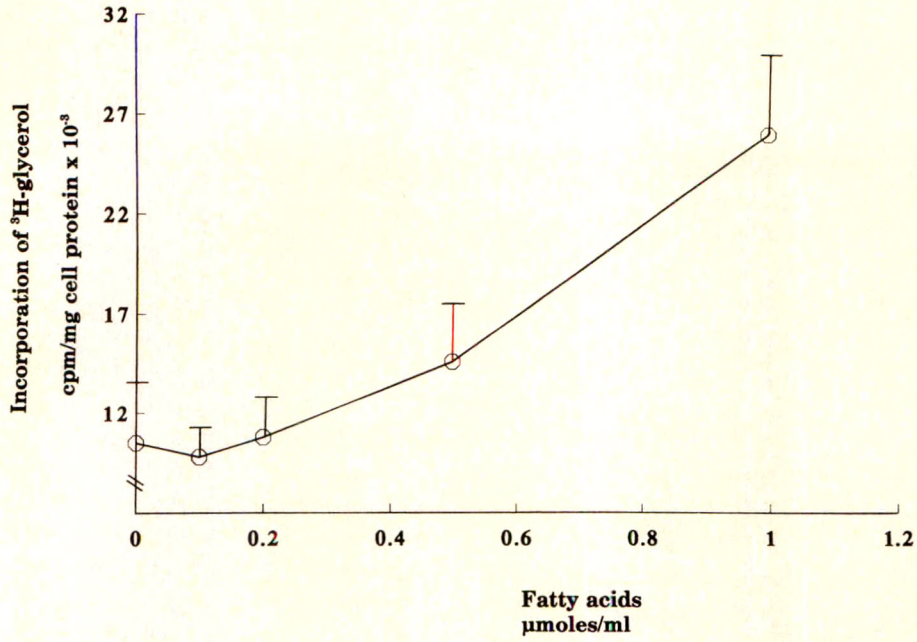


Fig. 6. Effect of fatty acids on incorporation of tritiated glycerol into endothelial triglycerides. Endothelial cells were exposed for 24 hours to sera from normally pregnant women with or without additional free fatty acids. Final concentrations of fatty acids in culture media are shown on *abscissa*. Each point represents mean ± SD of triplicate incubations.

responding increase in cytoplasmic lipid droplets was found (data not shown).

Release of free fatty acids as a result of enhanced lipolytic activity of the sera from women with preeclampsia could lead to a further increase in the ratio of free fatty acids to albumin. We therefore determined the lipolytic activity in sera from preeclamptic and normally pregnant women, and as shown in Fig. 7 the release of free fatty acids in the sera from women with preeclampsia was three to four times higher than in the sera from normally pregnant women ($p < 0.025$). Time-course studies showed linearity of the free fatty acid release up to 48 hours' incubation (data not shown). Eighty-five percent of this lipolytic activity could be inhibited by heat inactivation of both sera (data not shown). This procedure decreased markedly the incorporation of tritiated glycerol into endothelial triglycerides. However, a 100% higher tritiated glycerol incorporation into triglycerides was still present in endothelial cells exposed to heat-inactivated sera from women with preeclampsia as compared with heat-inactivated sera from normally pregnant women ($p < 0.01$) (Fig. 8).

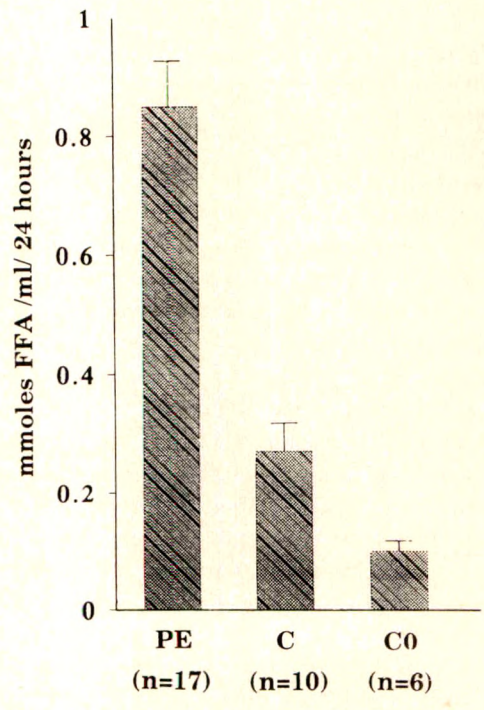


Fig. 7. Net increase in free fatty acids *(FFA)* in sera from preeclamptic women *(PE)*, from normal pregnancies *(C)*, or from nonpregnant women *(C0)*. A total of 50 µl of the various sera was incubated at 37° C for 24 hours, and increase in free fatty acid concentrations was measured. Numbers of individual sera and SD are indicated for each *bar*.

Comment

We recently reported that sera from women with preeclampsia induce accumulation of triglycerides in cultured endothelial cells.[5] In the current work we pro-

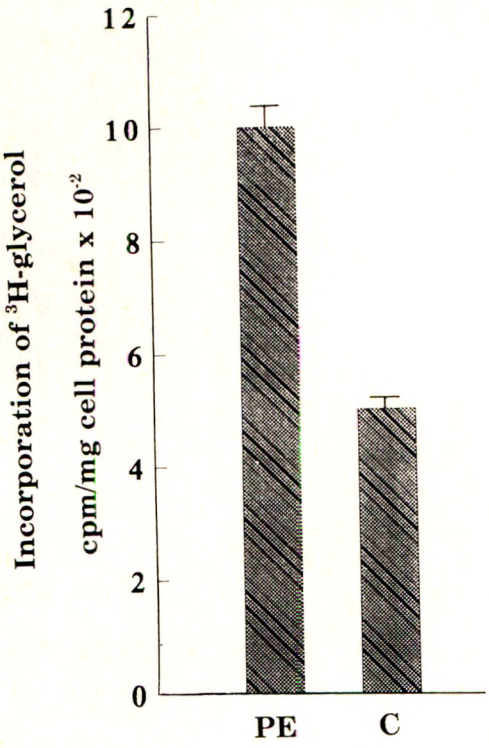


Fig. 8. Incorporation of tritiated glycerol into endothelial triglycerides after thermal treatment of sera. Endothelial cells were exposed for 96 hours to either sera from preeclamptic women *(PE)* or sera from normal pregnancies *(C)* that had previously been heat inactivated at 57° C for 60 minutes. Each *bar* represents mean ± SD of triplicate incubations.

vide strong evidence that the endothelial triglyceride accumulation is due to increased uptake of free fatty acids from the culture media containing sera from women with preeclampsia. We demonstrate that the endothelial triglyceride content increased by adding free fatty acids to the control sera. Conversely, by providing sera from women with preeclampsia with additional albumin the endothelial triglyceride accumulation was inhibited. Thus by altering the ratio of free fatty acids to albumin the degree of triglyceride accumulation in endothelial cells could be controlled. This is in accordance with the general conception that the major determinant of free fatty acid uptake in cells is the ratio of free fatty acids to albumin. Finally, we found only a minor effect of the triglyceride-rich very-low-density lipoprotein particles on the content of triglycerides in the endothelial cells.

The in vivo ratio of free fatty acids to albumin was higher in sera from preeclamptic women compared with controls. In experiments where the release of free fatty acids from the sera was minimized by heat inactivation of the lipolytic enzymes, a 100% higher incorporation of tritiated glycerol into endothelial triglycerides was still present after incubation with sera from women with preeclampsia compared with sera from normally pregnant women. In addition, the sera from women with preeclampsia contained an increased lipolytic activity that further amplified the ability of the sera to induce triglyceride accumulation in the endothelial cells.

Interactions of plasma lipids, lipoproteins, and the endothelial cells have been shown to be of major importance in the early development of atherosclerosis.[4] The endothelial cells can induce oxidative modifications of plasma low-density lipoprotein. Oxidized low-density lipoprotein is cytotoxic to endothelial cells and is taken up rapidly by macrophages, giving rise to foam cells.[4, 6] Also, remnants of triglyceride-rich lipoproteins are toxic to cultured endothelial cells.[10] Hyperlipidemia has been shown to inhibit endothelium-dependent relaxation of vasculature in vivo.[11] Furthermore, in vitro production of prostacyclin is influenced by both plasma lipoproteins and the types of fatty acids present.[12, 13] Thus there is ample evidence that the plasma lipids may interfere with the properties of endothelial cells.

Our previous work and the current study demonstrate that alterations in the lipolytic activity and the increased ratio of free fatty acids to albumin can change the properties of cultured endothelial cells. These findings have two aspects. First, the enhanced lipolytic activity of sera from women with preeclampsia further underscores that the lipid metabolism of women with preeclampsia is altered. Second, the free fatty acids and possibly other lipid or enzymatic components of the sera from women with preeclampsia may be mediators in inducing endothelial dysfunction in preeclampsia.

To further test "the lipid hypothesis of preeclampsia" studies are in progress to characterize the enhanced lipolytic activity of sera from women with preeclampsia.

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Use of the fetal chest in estimating fetal weight

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OBJECTIVE: This study was designed to develop formulas using the chest circumference instead of the abdominal circumference for estimating fetal weight.

STUDY DESIGN: Ultrasonographic measurements of the chest circumference, biparietal diameter, abdominal circumference, humeral length, and femoral length were obtained in 75 term fetuses of uncomplicated pregnancies within 24 hours of delivery. Three equations for fetal weight estimation that used the chest circumference, instead of the abdominal circumference, in combination with the biparietal diameter or the humeral length were developed by regression analysis.

RESULTS: The average mean errors of fetal weight estimation for these equations vary from 7.1% to 7.6%.

CONCLUSIONS: These equations may be used in predicting the birth weight when the fetal abdomen is altered by certain fetal abnormalities. (Am J Obstet Gynecol 1992;167:448-50.)

Key words: Fetal chest, fetal weight

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Estimating the fetal weight by ultrasonography on the basis of computer-generated equations has been useful in the clinical management of many obstetric conditions. Equations that use the abdominal circumference (AC) in combination with the head circumference (HC), the biparietal diameter (BPD), or the femoral length (FL) have been extensively used for this purpose.[1-3] Certain fetal conditions such as abdominal wall defects, significant uropathy, and intestinal ob-

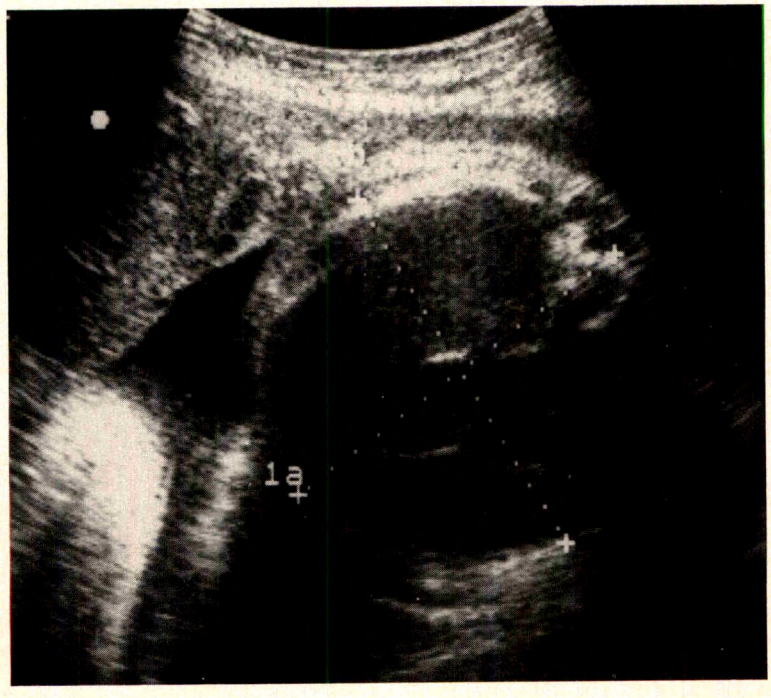


Fig. 1. Fetal chest circumference is obtained with two perpendicular diameters measured at four-chambered view of heart along bony rib margins.

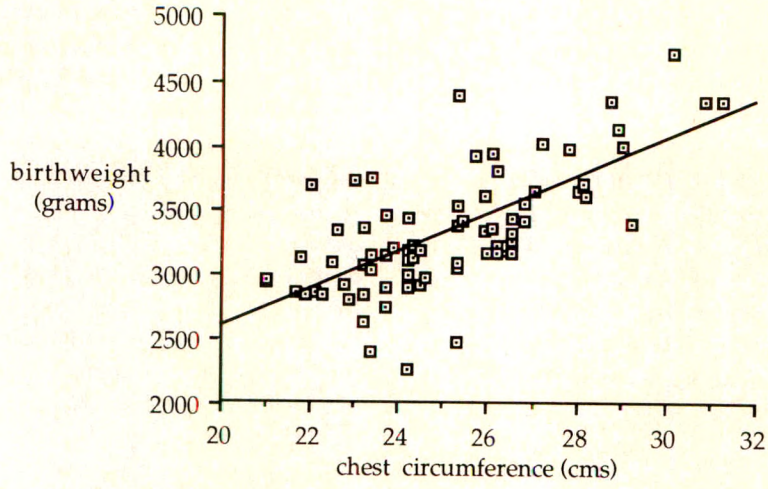


Fig. 2. Scattergram along with linear regression line of birth weight versus fetal chest circumference is based on 75 patients.

Table I. Equations for estimating fetal weight (N = 45 patients)

Equation No.	*Coefficient correlation*	*p Value*
1. BWT = 154(CC) + 420(BPD) − 4507	0.86	0.0001
2. BWT = 148(CC) + 458(HL) − 3509	0.87	0.0001
3. BWT = 138(CC) + 320(HL) + 280(BPD) − 4908	0.88	0.0001

Table II. Percentage error of fetal weight estimation (N = 30 patients)

Equation No.	*Mean*	*SD*	*p Value*
1	7.6	8.8	—
2	7.4	8.8	—
3	7.1	7.6	—
4 (1)	1.0	9.8	0.001
5 (2)	2.1	11.1	0.001

Percentage error = 100 (EFW − BWT)/BWT. The p values are calculated for the difference in mean percentage errors between equation 4 or 5 and equation 1, 2, or 3.

Table III. Accuracy of fetal weight estimation (N = 30 patients)

Equation No.	*≤5% of BWT*	*≤10% of BWT*	*≤15% of BWT*
3	40	57	70
4	33	77	87
5	33	73	87

struction or atresia may make the AC unsuitable for an accurate estimation of the fetal weight. This study was designed to develop formulas that use the chest circumference (CC) instead of the AC for estimating fetal weight.

Material and methods

This study was carried out at the Washington University Medical Center during the period from Aug. 1, 1989, to Oct. 30, 1989. Ultrasonographic measurements of BPD, HC, AC, CC, FL, and humeral length (HL) were made in 75 term fetuses of uncomplicated pregnancies within 24 hours of delivery with the use of a 3.5 MHz linear transducer. The BPD was measured at the level of the midbrain from the outer margin to the inner margin of the proximal and distal skull tables respectively. The femoral length was measured from the greater trochanter to the lateral epicondyle. The chest circumference was measured in a transverse plane at the level of the four-chambered view of the heart along the bony margins of the chest (Fig. 1). Anteroposterior (AP) and transverse (TRV) diameters were measured and the circumference was calculated according to the formula: Circumference = (AP + TRV) × 1.57. The relationship between ultrasonographic measurements (BPD, HC, CC, AC, FL, and HL) and birth weight (BWT) was retrospectively examined by stepwise regression analysis based on 45 patients. Equations for estimating the fetal weight by means of the chest circumference with the best correlations were developed. These equations were then prospectively used to estimate the fetal weight in 30 patients. For comparison, the estimated fetal weights were also obtained from the following previously published equations that utilize the AC: Log 10 BWT =

$-1.7492 + 0.166(BPD) + 0.046(AC) - 2.646(AC + BPD)/1000$ and Log 10 BWT $= 1.3598 + 0.051(AC) + 0.1844(FL) - 0.0037 (AC \times FL)$. These two equations are from references 1 and 2 and are identified as equations 4 and 5, respectively, in our subsequent discussion and in Tables II and III. Relative error was calculated by dividing the difference in the estimated fetal weight (EFW) and the BWT by the BWT. Underestimation of the birth weight results in a negatively relative error. The Student *t* test was used to compare the mean percentage errors of estimating fetal weight by our equations with those by equations 4 and 5. A *p* value of <0.05 is considered statistically significant.

Results

Birth weights range from 2260 to 4740 gm. The chest circumference varies linearly with the birth weight with a correlation coefficient of 0.69 and a *p* value of 0.001 (Fig. 2). The three best-fit equations that used CC in combination with BPD, HL, or FL are presented in Table I. The correlation coefficients of these equations are 0.86, 0.87, and 0.88, respectively. The accuracy of predicting the birth weight is summarized in Tables II and III. Table II shows the means and the standard deviations of the systematic percentage error. Table III shows the percentage of patients with the estimated fetal weight within 5%, 10%, or 15% of the birth weight, respectively. The equations containing CC tend to underestimate the birth weight. The difference in the relative mean percentage error of predicting BWT between the equations that use CC and those using AC as proposed by Shepard et. al.[1] or Hadlock et al.[2] is statistically significant (Table II). The intraobserver variation coefficient for measuring the chest circumference is 5%.

Comment

Chest circumference linearly increases while the ratio of the chest circumference to the abdominal circumference remains constant at about 0.89 as gestation advances during the interval of 16 to 40 weeks.[4] The chest circumference increases on an average 9.1 mm and 6.3 mm per week during the intervals of 20 to 30 weeks and 30 to 40 weeks, respectively.[5] Our study demonstrated that the chest circumference correlates proportionately with the birth weight in normal term pregnancies. In addition, the mean percentage error of fetal weight estimation with the CC, HL, and BPD as in our equation 3 is 8.1%, which is comparable to that reported by others using the AC.[1, 3] Fifty-seven percent of our patients have the estimated fetal weight within 10% of the birth weight. Similarly, Shepard et al.[1] reported that the fetal weight could be predicted within 10% of the birth weight with the use of the computer-generated formula and both the AC and BPD in 54.8% of their patients. Our study demonstrates that the equations that use the AC are more accurate in predicting the birth weight than those that use the CC for the same study population. Thus the previously published equations that use the AC in combination with BPD, FL, or both are preferable to ours in estimating fetal weights when AC can be accurately measured in term pregnancies. However, our equations could be reasonable alternatives when the measurement of the AC may not be optimal for the purpose of fetal weight estimation. The latter may occur in fetal conditions such as abdominal wall defects, obstructive uropathy, or bowel obstruction. The increased fetal abdominal circumference in these situations may not necessarily reflect the increased fetal weight.

Thus our equations that use the CC in combination with the BPD or HL can be used to estimate fetal weight with a high degree of accuracy. These equations may be helpful in selected cases of fetal abnormalities.

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Oral condyloma lesions in patients with extensive genital human papillomavirus infection

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OBJECTIVES: The incidence, location, and morphologic appearances of human papillomavirus oral lesions in patients with genital condylomatosis were investigated with clinical, colposcopic, and histologic examination as diagnostic procedures. The human papillomavirus types were also evaluated with filter in situ hybridization.

STUDY DESIGN: One hundred one patients, 66 female and 35 male, with genital condyloma underwent an oral cavity examination. Ninety-nine (99%) practiced orogenital sex, and all were asymptomatic for oral lesions.

RESULTS: Ninety-one underwent biopsy; histologic studies gave a diagnosis of condyloma in 48% of 101 specimens collected. In 8 of 91 (9%) oral lesions were suspected by naked-eye examination; they were confirmed histologically in all eight. Of 83 patients suspected of having oral condyloma on colposcopic examination, 38 (46%) were confirmed histologically. Thus 38 of 46 patients (83%) had oral condyloma not visible to the naked eye. Colposcopically, oral lesions appeared filiform (50%), moruloid (26%), and mixed (24%). Twenty cytologic oral samples were also collected for deoxyribonucleic filter in situ hybridization analysis. Human papillomavirus deoxyribonucleic genital types were observed in 45% (9/20) of all oral scrapings collected, and all were histologically confirmed.

CONCLUSION: Our data indicate that genital human papillomavirus types are capable of establishing a local infection in the oral cavity and demonstrate a high incidence of human papillomavirus oral lesions in patients with genital condyloma. (Am J Obstet Gynecol 1992;167:451-8.)

Key words: Oral condyloma lesions, human papillomavirus, colposcopy

In the oral cavity the presence of various clinical expressions of different human papillomavirus (HPV) types has been reported by several authors.[1-5] In particular HPV type 13 has been found to be exclusively associated with focal epithelial hyperplasia.[6, 7] Moreover, recently the presence of HPV types 6, 11, 16, and 18 has also been found in different oral lesions.[8-11] However, the prevalence and clinical spectrum of HPV oral cavity infection and the oncogenic potential of these lesions are still unknown. The manner of HPV transmission into the oral cavity is not precisely known. Direct contact and self-inoculation are probably the main contributors to the diffusion of the disease in sexually active individuals.[12-14]

In this study we wanted to investigate the presence, location, and morphologic appearances of HPV oral lesions in patients affected by genital condylomatosis by means of clinical, colposcopic, and histologic examination as diagnostic procedures. A series of 20 patients were also evaluated for the presence of HPV types in oral cavity lesions with filter in situ hybridization.

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Material and methods

From February 1988 through February 1990, 101 unselected, consecutive patients attending the Colposcopy Clinic of the Department of Gynecology of the Catholic University were evaluated and enrolled for an oral cavity examination. All patients gave consent to participate in the study, and no one refused an oral cavity inspection with histologic examination. The study group consisted of 66 female (median age 33 years, range 18 to 45) and 35 male (median age 32 years, range 24 to 45) subjects with histologically confirmed genital condyloma. All patients were asymptomatic. The median age of the first coitus was 16 years (range 15 to 22), and the median number of partners was four (range 1 to 15). Ninety-nine of 101 patients (98%) practiced orogenital sex, and all showed one or more genital sites affected by HPV. In particular female patients showed vulvar (100%), cervical (56%), urethral (48%), vaginal (45%), and perianal and anal (38%) HPV lesions, whereas male patients showed HPV lesion of

Table I. Oral colposcopic lesion characteristics in 101 patients subjected to oral cavity examination

No of patients with suspected oral lesions	91
Oral site affected (%)	
Tongue	100
Buccal mucosa	2
Lip	1
Tongue sites affected (%)	
1	76
2	22
>2	2
No and size of lesions (%)	
>5 Lesions	61
<5 Lesions	39
>2 mm	58
<2 mm	42
Morphologic type (%)	
Filiform	50
Moruloid	26
Mixed	24

the penile prepuce (87%), the penile shaft (25%), the glans with corona (35%), and the urethra meatus (3%).

The epithelial surface of the oral cavity was inspected visually and by colposcopic examination with a Opm1 CD PLUS Zeiss colposcope (Zeiss, Oberkochen Germany).

Excisional biopsies under colposcopic guidance were taken from suspected HPV oral lesions. Specimens were placed in formalin for routine histologic studies. Cytologic oral samples were collected from the last 20 patients with cotton exfoliating swabs before biopsy, and samples were put in phosphate-buffered saline solution and stored at −20° C until assayed by filter in situ hybridization technique.

Clinical and colposcopic terminology. After application of a 5% acetic acid solution in the oral mucosa and in the back and bottom of the tongue, which is left in place for 2 to 3 minutes, it is frequently possible to reveal the presence of white areas and colposcopic lesions similar to those observed in the genital tract. The following types of characteristic colposcopic lesions were observed: (1) white filiform lesions with an evident capillary loop (2) white, roundish lesions with a central capillary loop and irregular surface (like a microscopic mulberry), which we defined "moruloid" and (3) shiny white keratotic plaques.

Because of some morphologic similarites to HPV-related lesions of the genital tract, we hypothesized that these lesions could be indicative of HPV infection of oral cavity.

We recorded all lesions ≤2 mm or >2 mm in the two major perpendicular diameters and also the number of lesions more than five or less than five in number for each oral area affected.

Histologic terminology. Typical oral condyloma had histologic features similar to those found in the genital area, where squamous epithelium with mild infiltration of lymphoid cell is present. The epithelium showed acanthosis (increased thickness of the spinous layer), hyperkeratosis (increased thickness or density of the stratum corneum), and koilocytosis (normal to enlarged hyperchromatic mononuclear or binuclear cells with perinuclear cytoplasmic vacuolization). The presence of multiple koilocytosis was taken as the pathognomonic criteria to classify the lesion as condyloma. Therefore, lesions showing only minimal histologic changes, i.e., a mild degree of acanthosis and hyperkeratosis without koilocytosis or focal isolated koilocytotic changes, were not classified as condyloma.

Filter in situ hybridization. Filter in situ hybridization technique was performed according to the method described by Wagner et al.[15]

By means of a counting chamber, the number of cells was estimated to be about 1 × 10 E4 epithelial cells for each sample. After thawing, the cells were resuspended and filtered into a nitrocellulose filter (0.45 μm pore size, 25 mm diameter, Sartorius Filters Inc., Hayward, Calif.).

Cell lysis and deoxyribonucleic acid (DNA) denaturation were performed by overlaying the filter on a Whatman 3 MM paper, which was soaked in 1.5 mol/L sodium chloride and 0.05 mol/L sodium hydroxide for 5 minutes. Filters were then placed twice for 10 minutes on a Whatman 3 MM paper previously soaked in 0.5 mol/L Tris, pH 7.4, 20× saline sodium citrate (3 mol/L sodium chloride and 0.3 mol/L sodium citrate). Filters were air dried and baked at 80° C for 30 minutes to fix the DNA . Each filter was cut in half and stored in 50 ml plastic Falcon tubes (approximately 40 half filters per tube) until further processing.

The filters were prehybridized for 4 hours at 42° C. The prehybridization solution consisted of 50% formamide, 5× saline sodium citrate, 50 mmol/L sodium phosphate buffer (pH 6.5), 0.02% bovine serum albumin, 0.02% Ficoll 400, 0.02% polyvinylpyrrolidone, 0.05 mg/ml denatured transfer ribonucleic acid (Boehringer Mannheim, Germany).

Hybridization was performed for 40 hours under stringent conditions at 42° C, which corresponded to 20° C below melting temperature (−20° C). The solution was the same as above; however, the concentration of transfer ribonucleic acid was modified (0.1 mg/ml) with the addition of 2 × 10 E6 counts/min/ml of denatured phosphorus 32–labeled HPV DNA probe.

After hybridization the filters were washed three times for 30 minutes in 2× SCC containing 0.1% sodium dodecyl phosphate at 68° C.

The most filters were placed in stampholders and exposed to Kodak (Eastman Kodak Co., Rochester, N.Y.) X-Omat radiographic film for 5 days at −70° C.

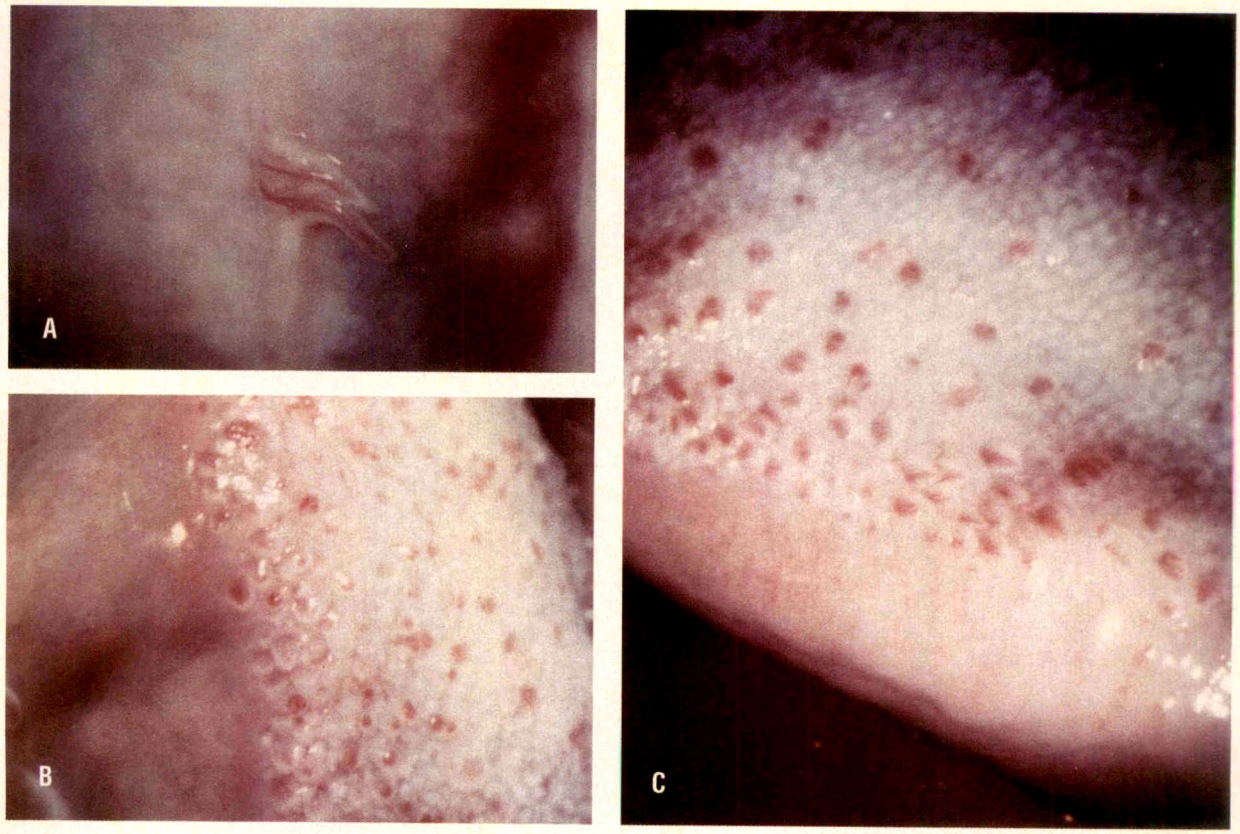


Fig. 1. Morphologic features of condylomatous lesions of tongue at colposcopy. **A,** Filiform lesions of lower surface of tongue. White wart with central capillary loop is evident. (Original magnification ×12.) **B,** Some moruloid lesions of lateral border of tongue. (Original magnification ×12.) **C,** Both filiform and moruloid lesions are evident in this patient with oral HPV infection. (Original magnification ×12.)

Probe DNA and radiolabeling. Recombinant plasmid containing types 6, 11, 16, and 18 DNA were kindly provided by Dr. E. M. de Villiers (Deutsches Krebsforschung Zentrum, Heidelberg).

The HPV DNA recombinant clones were amplified by standard methods.[16] The HPV DNA insert was separated from the vector by restriction enzyme digestion, electrophoresis, and electroelution and was subsequently purified over NACs Prepac columns (BRL, Gaithersburg, Md.) as recommended by the manufacturer. The DNA was labeled with ^{32}P-deoxythymidine (New England Nuclear, DuPont) by nick translation to a specific activity of >10 E8 counts/min/μg of DNA.[17]

The filter in situ hybridization was performed with two mixtures of HPV types 16 and 18 DNA and HPV types 6 and 11 DNA, respectively.

Results

Oral lesion characteristics are shown in Table I. Of 101 patients with extensive genital HPV infection, 91 (90%) were found to have suspicious oral lesions. In all suspected patients the tongue was affected, and the lesions were located on lateral borders and lower and back surface (Fig. 1). Colposcopic findings of the tongue consisted of filiform (Fig. 1, *A*) or moruloid lesions (Fig. 1, *B*), whereas both types of lesion can be observed in the same patient (Fig. 1, *C*).

All patients with suspected oral condyloma lesions underwent biopsy. Seventy-five patients had a single biopsy, and 16 had two biopsies, for a total of 107 specimens. The histologic examination of the 107 specimens collected gave a diagnosis of condyloma in 49 out of 101 (48%) specimens. In six cases (6%), specimens were not sufficient for a diagnosis. In the remaining 52 specimens, histologic studies gave a diagnosis of papillomatosis in 10%, leukoplakia in 8%, hyperkeratosis in 7%, mixed in 10%, and normal tissue in 10%. No intraepithelial neoplasia was detected. Morphologically, the 107 specimens collected for histologic examination appeared as filiform lesions in 64 cases (60%), as moruloid lesion in 28 cases (26%), as leukoplakia in only one case (0.9%), and as mixed lesions in the remaining 14 cases (13%). Moruloid and filiform colposcopic lesions showed different histologic features; in the first form (Fig. 2, *A* and *B*) the epithelial lining surface was flat, whereas in the latter (Fig. 3, *A* and *B*) the epithelium covered dermal fibrovascular axes in fine digita-

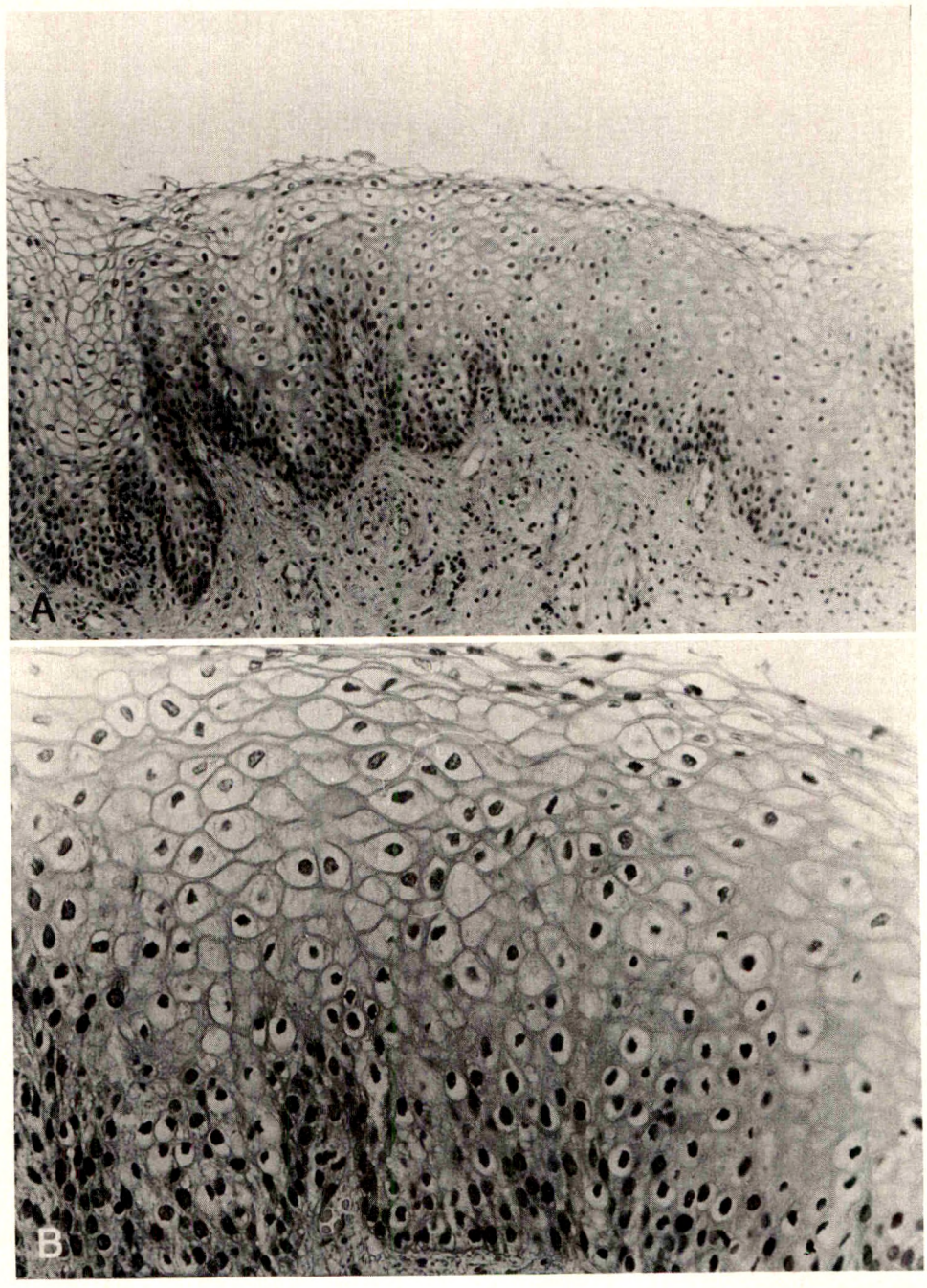


Fig. 2. A, Photograph shows epithelial lining with flat surface. There is a mild degree of papillomatosis and a moderate flogistic infiltrate in chorion. (Original magnification ×250.) **B,** Higher magnification showing epithelial koilocytotic changes. (Original magnification ×400.)

tions. Eight of 91 patients (9%) were found to have suspicious oral lesions by visual inspection, and all were confirmed histologically. Among 83 patients colposcopically suspected for oral condyloma, 38 (46%) were histologically confirmed; thus 38 of 46 patients (83%) had oral condyloma not visible to the naked eye.

Among 48 histologically confirmed diagnoses of tongue condyloma, 17% were located in the lower surface, 71% on the lateral borders, and 13% on the dorsum of the tongue. Histologic studies gave a diagnosis of condyloma in one of three biopsy specimens taken from lip and oral mucosa. Table II also shows the correlations between morphologic type, location of colposcopic lesions, and histologic type. The lateral borders of the tongue showed only filiform warts in 70% (42/60) of sites, and 66.6% of these were histologically confirmed. Most of remaining cases showed mixed morphologic types. On the other hand, all lesions lo-

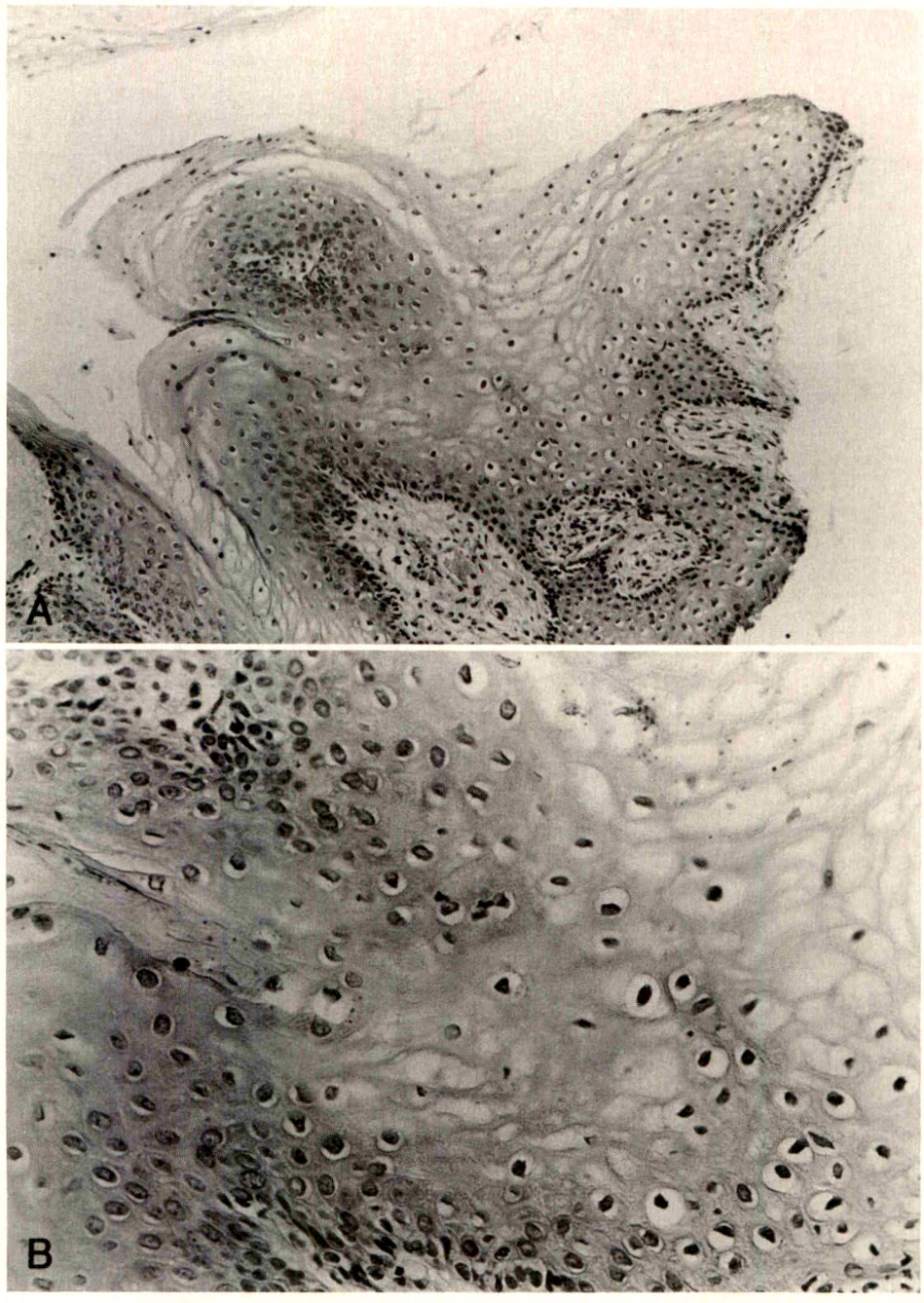


Fig. 3. A, Fine extroflexion composed of thin fibrovascular axes lined by squamous epithelium in stroma. There is a slight infiltration of parvicellular elements. (Original magnification ×250.) **B,** Higher magnification showing koilocytotic changes. (Original magnification ×400.)

cated on the dorsum of the tongue had a moruloid surface and were histologically confirmed as condyloma in 27.2% of cases.

The correlation between the colposcopic features and the histologic findings is reported in Table III. In particular, koilocytes were more frequently present in filiform lesions than in moruloid lesions.

Finally, to confirm the relationship between genital and oral condyloma lesions, cytologic oral samples obtained from the last 20 patients observed were analyzed for the presence of genital type HPV DNA by filter in situ hybridization (Table IV). HPV DNA were found in nine of 20 (45%) different patients. In five (56%) of the HPV-positive patients the viral sequences were homologous to those of HPV types 6 through 11, and in three (33%) they were homologous to types 16 through

Table II. Correlation between morphologic type location of colposcopic oral cavity lesions, and histologic type

Morphology	*Oral sites*	*No.*	*%*	*Condyloma*	
				No.	*%*
Tongue					
Filiform	Lateral borders	42	42.4	28	66.6
Filiform	Lower surface	17	17.7	8	47.0
Moruloid	Dorsum	22	22.2	6	27.2
Moruloid	Lateral borders	3	3.3	1	33.3
Filiform and moruloid	Lateral borders	15	15.5	5	33.3
Others					
Moruloid	Lip	1	33.3	—	—
Leukoplakia	Oral mucosa	2	66.6	1	50.0

Table III. Correlation between colpscopic appearance and histologic type

Colposcopic appearance	*No. Tot.*	*Histologic type*			
		Papillomatosis		*Koilocytosis*	
		No.	*%*	*No.*	*%*
Filiform	69	8	12	41	59
Moruloid	30	2	7	7	23
Leukoplakia	2	—	—	1	50

18. In one case viral DNA homologous to that of types 6, 11, 16, and 18 was present. In the same 20 patients cytologic cervical samples were also collected. Among these 12 samples were positive for HPV types, with a concordance rate between cervical and oral samples of 80%. Finally, the correlations between the sites of tongue localization of lesions and HPV DNA type in the nine patients with oral HPV infection are shown in Table V.

Comment

Our study shows that 50% of patients with multiple and widespread genital HPV infection who practice orogenital sex had histologically confirmed oral condylomas. This high incidence in a large series of patients, all asymptomatic for oral lesions, has never been reported in literature, although in the last years an increasing interest in HPV research by oral pathologist has been reported, and the genital oral sexual transmission of the organism has been hypothesized.[9, 11, 13, 14]

Our results may be related to the particular sexual behavior of patients analyzed, who practiced orogenital sex. The simultaneous cytologic detection of the same HPV types both in the oral cavity and the genital tract of patients supports this mode of transmission. However, the systematic use of colposcopy as a diagnostic procedure must also explain the higher incidence of oral HPV lesions in our study, compared with other studies in which colposcopy was not performed.[7, 10, 11, 13]

In fact, the much higher sensitivity of colposcopy with respect to simple visual examination (83% vs 9%) suggests that magnification is a mandatory procedure for detecting oral lesions. Colposcopy also allows us to establish the exact location, number, and morphologic appearance and to perform direct biopsies of the oral areas suspected. In contrast to others authors who reported oral condylomatous lesions equally distributed among various oral cavity sites,[14] in our series the tongue represents the oral site most frequently affected. In our series oral condylomas appeared as multiple, small, white or pink nodules, sessile or pedunculate with a moruloid aspect, and as papillary growth with filiform characteristics. The size of oral lesions was >2 mm in >50% of lesions, and in 61% of cases more than five lesions were present. Therefore our data confirm and extend previous observations obtained in small series of patients.[9, 11, 14]

Histologic examination gave a diagnosis of oral condylomas in 48% of 101 biopsies collected, whereas 27% of the remaining negative specimens showed features usually indicative of the presence of HPV infection.[21] The histologic finding of normal tissue in 10% of our biopsy material could be ascribed at least in part to an incorrect sampling.

HPV types 6, 11, and 13 have been identified in warty lesions of the oral cavity.[5, 22] HPV type 16 has also been found, but it is less commonly associated with benign oral lesions.[5] The results reported in our study demonstrate that HPV types 16 and 18 and 6 and 11, which

Table IV. Clinical, colposcopic and histologic characteristics of HPV typed lesions

Subject No.	*Sex*	*Age (yr)*	*HPV type**	Colposcopic appearance	Histologic type
1	M	29	6/11	Filiform	Widespread koilocytosis
2	M	29	Negative	Filiform	Papillomatosis with koilocytosis
3	F	33	Negative	Filiform	Hyperkeratosis with koilocytosis
4	M	33	Negative	Filiform	Papillomatosis with koilocytosis
5	M	45	6/11	Filiform	Papillomatosis with koilocytosis
6	F	28	16/18	Filiform	Papillomatosis with koilocytosis
7	F	24	Negative	Filiform	Papillomatosis with koilocytosis
8	F	44	6/11	Filiform	Condyloma acuminatum
9	F	30	6/11	Filiform	Condyloma acuminatum
10	F	30	Negative	Filiform	Condyloma acuminatum
11	F	29	Negative	Filiform	Papillomatosis with koilocytosis
12	F	23	Negative	Filiform	Papillomatosis with koilocytosis
13	F	28	6/11, 16/18	Filiform	Condyloma acuminatum
14	F	23	Negative	Filiform	Condyloma acuminatum
15	F	40	Negative	Filiform	Condyloma acuminatum
16	M	38	Negative	Filiform	Papillomatosis with koilocytosis
17	F	25	Negative	Filiform	Widespread koilocytosis
18	F	26	16/18	Filiform	Papillomatosis with koilocytosis
19	F	20	16/18	Moruloid	Condyloma
20	F	35	6/11	Filiform	Papillomatosis with koilocytosis

*Filter in situ hybridization was performed with two mixture of HPV types 6 and 11 DNA and HPV types 16 and 18 DNA, respectively.

Table V. Correlation between age, sex, localization of lesions, and HPV DNA type in nine patients with oral condyloma

Age (yr)	*Sex*	*Tongue site of lesions*	*HPV types**
29	M	Lateral borders	6-11
45	M	Right lateral border	6-11
28	F	Left lateral border	16-18
20	F	Dorsum	16-18
26	F	Lower surface and lateral borders	16-18
44	F	Dorsum and left lateral border	6-11
30	F	Lateral borders and lower surface	6-11
35	F	Right lateral border	6-11
28	F	Lateral borders	6-11, 16-18

*By filter in situ hybridization technique (see Material and methods).

are frequently found to be associated with genital lesions, can be also detected in cells obtained from oral condyloma with filter in situ hybridization. Moreover, the incidence of lesions positive for these HPV types in the oral cavity resembles the one previously demonstrated for the genital condyloma.

In fact, HPV DNA was observed in 45% (9/20) of all oral scrapings examined, and all were histologically confirmed. It is interesting to note here that the greater number of positive samples for HPV types 6 and 11 (55%) compared with those which had DNA homologous to types 16 and 18 (33%). These data might have a prognostic significance for these patients. Moreover, the presence in the oral cavity of HPV types 16 and 18, which are more frequently associated to malignant genital diseases, point to the importance of having a simple and noninvasive technique that allows screening and follow-up of a high number of subjects in whom HPV infection is suspected. On the basis of this report a large clinical trial is now in progress in our department, aimed at comparing the occurrence of HPV subtypes in simultaneous biopsies taken from different areas of the genital tract and oral cavity from patients with histologically confirmed genital and oral condyloma.

The presence of genital types of HPV DNA in cells obtained from oral condylomatous lesions indicates that these viruses are capable of establishing a local infection in different sites of the oral cavity. From our data and in agreement with other authors,[5, 20] it should be hypothesized that primary oral infection can follow a direct contact between virus and oral epithelium, as suggested by the sexual behavior of the patients studied, whereas secondary infections may result from hematogenous spread or autotransmission in patients with a HPV genital infection.[4, 5]

In conclusion, the presence of genital condylomas should alert the clinician to inquire about the presence

of simlar wartlike lesions in the oral cavity, with particular regard to those patients who practice orogenital sex. Moreover, colposcopy appears to be an invaluable aid in establishing the diagnosis of oral condyloma.

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A comparison of circulating hormone levels in postmenopausal women receiving hormone replacement therapy

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Seventeen postmenopausal subjects were randomized into this comparative study of esterified estrogen 0.625 mg (Estratab), esterified estrogen 0.625 mg plus 1.25 mg methyltestosterone (Estratest H.S.), or conjugated estrogen 0.625 mg (Premarin). Sixteen subjects completed the study in which plasma hormone concentrations of estrone and estradiol were assessed at various time points. There were no significant differences among the treatment groups. (Am J Obstet Gynecol 1992;167:459-60.)

Key words: Hormone levels, postmenopausal women, hormone replacement therapy

There are many different products available to clinicians for the treatment of postmenopausal symptoms, as well as a number of therapeutic regimens.[1] Each product has been shown to have particular benefits and allow for individual needs and concerns of patients, as well as their physicians. Management of postmenopausal symptoms usually includes oral estrogen, progestogen, and androgen formulations currently marketed. Whereas a number of estrogen formulations have been prescribed for decades, there is little comparative information available on the differences in potencies and pharmacologic effects. Some laboratories have measured free and total plasma estradiol and estrone levels during a 25-day administration of estrone sulfate and estradiol. Others have correlated estrogenic potency with hepatic protein production after administration of various oral, transdermal, and vaginally delivered estrogen preparations.[2]

The purpose of this study was to measure and compare plasma levels of estrone and estradiol in postmenopausal women after the administration of commercially available oral esterified or conjugated estrogen products.

Patients and methods

The investigation was approved by the local human investigation committee and informed consent was obtained. Seventeen postmenopausal subjects (14 natural and three surgical) entered the study. The demographic characteristics were similar across the three treatment groups. Mean age was 53.95 years with a range of 37.9 to 65.8 years. Two patients presented with menopausal symptoms at screening. At baseline (after washout) six patients were experiencing menopausal symptoms. Seventy-one percent of the subjects were white and 29% were categorized as other (Hispanic, $n = 3$; black, $n = 1$). Subject 60186 did not meet inclusion-exclusion criteria and was dropped from the study shortly after treatment was begun.

After patient randomization, blood samples were taken from patients at baseline and 8 hours after the first dose of drug. Patients returned to the clinic weekly for 5 weeks while taking medication at the same time daily after their morning meal. Blood samples were drawn 8 ± 2 hours after the dose of study medication at each clinic visit for assay of plasma hormone levels. The plasma follicle-stimulating hormone level was de-

Table I. Levels of follicle-stimulating hormone

Subject identifier	*Treatment group*	*Follicle-stimulating hormone (mIU/ml)*	
		Screening	*Day 29*
60178	Estratab	99.6	*
60180	Estratab	82.3	45.3
60183	Estratab	65.8	54.0
60188	Estratab	64.9	59.3
60190	Estratab	73.2	40.3
60193	Estratab	78.3	57.8
60177	Premarin	60.2	22.2
60182	Premarin	91.6	63.1
60184	Premarin	157.4	83.1
60187	Premarin	109.1	66.9
60189	Premarin	128.6	83.9
60179	Estratest H.S.	151.1	86.1
60181	Estratest H.S.	89.4	73.3
60185	Estratest H.S.	143.3	69.1
60191	Estratest H.S.	109.3	62.9
60192	Estratest H.S.	141.6	87.4

*Data not available.

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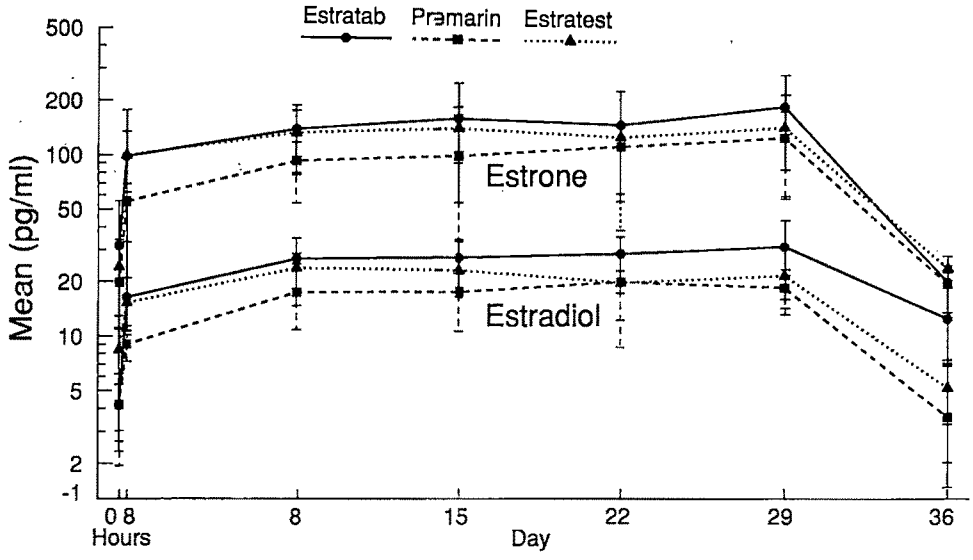


Fig. 1. Mean estradiol and estrone levels over time.

termined at screening and on day 29. One week after administration of the last dose of study drug (day 36), patients returned to the clinic for final evaluation and blood drawing. Estrone, estradiol, and follicle-stimulating hormone were measured by radioimmunoassay (Nichols Laboratories, San Juan Capistranc, Calif.).

Results

Sixteen subjects completed the study: six in the Estratab group, five in the Estratest H.S. group, and five in the Premarin group. There were no significant differences between the treatment groups in the plasma concentrations of estrone and estradiol measured at any of the time points. The mean change in follicle-stimulating hormone from screening to day 29 was clinically insignificant among the three treatment groups (see Table I for subject levels). Vital signs remained constant over the course of the study.

Comment

As can be seen in Fig. 1, estrone and estradiol levels were fairly constant during treatment. Similar blood concentration levels appeared to be reached after a week of treatment with all three products. Circulating estrogens were cleared at the same rate after the end of treatment with all products and levels returning to approximately baseline levels within 7 days. The biologic action on the ovarian-pituitary feedback loop was apparently equivalent for all products, as they all appeared to suppress follicle-simulating hormone similarly after treatment with the study drug.

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Prenatal diagnosis of human parvovirus B19 in nonimmune hydrops fetalis by polymerase chain reaction

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OBJECTIVE: Nonimmune hydrops fetalis is a potentially lethal condition reflecting the clinical manifestation of several pathologic processes. Recently maternal infection by human parvovirus B19 has been reported to result in nonimmune fetal hydrops. We sought to develop a rapid and sensitive test to detect the presence of this agent in utero.

STUDY DESIGN: Using a cloned isolate of the virus, we developed an assay based on enzymatic amplification of a segment of the human parvovirus B19 genome that allows direct detection of this agent in samples of fetal blood and amniotic fluid.

RESULTS: The method detected as few as 100,000 genome equivalences and was specific for the viral genome alone. We used this assay to evaluate nine fetuses initially seen with nonimmune hydrops. Three cases were found to be positive for the human parvovirus B19 genome.

CONCLUSION: The method is powerful in that it is rapid, sensitive, and simple. This assay may have general applicability in evaluation of nonimmune hydrops and in documentation of the natural history of fetal human parvovirus infections. (AM J OBSTET GYNECOL 1992;167:461-6.)

Key words: Prenatal diagnosis, parvovirus B-19, hydrops fetalis, polymerase chain reaction

Hydrops fetalis is a potentially lethal condition that can be caused by immunologic or nonimmunologic factors. The advent of measures to prevent rhesus factor isoimmunization has resulted in nonimmune fetal hydrops becoming the most frequently encountered form of this condition in the United States.[1] It is estimated that this condition complicates 1 in every 3000 to 4000 pregnancies.[2]

Importantly, a variety of conditions have been associated with nonimmune fetal hydrops. Therefore depending on the cause the fetal outcome can be quite variable. In some cases the hydrops appears to resolve spontaneously, resulting in an apparently normal offspring. However, in other cases the prognosis for the fetus is grave.[3] When fetal hydrops is diagnosed by ultrasonography a clinical dilemma arises with respect to the most appropriate counseling and management. Thus it is important that as much information as possible be gathered relative to the cause so that the antenatal management of this disorder is optimal.[4]

Recently maternal infection by human parvovirus B19 during gestation has been reported to be associated with nonimmune fetal hydrops.[5] Therefore in addition to other modalities of diagnosis a rapid test to detect the presence of human parvovirus B19 in utero would add to the clinical tools used in the evaluation of nonimmune hydrops and aid the clinician in management decisions. In this study we report on a sensitive method to detect the presence of the human parvovirus B19 deoxyribonucleic acid (DNA) in clinical specimens based on sequence-specific DNA amplification.[6] This polymerase chain reaction detection method was used to identify a portion of the parvovirus B19 genome in samples of fetal blood and amniotic fluid obtained from several pregnancies presenting with nonimmune hydrops fetalis.

Material and methods

A 20-base oligonucleotide probe complimentary to nucleotides 1730 through 1749 of a human parvovirus B19 isolate[7] was synthesized on an automated DNA synthesizer (model 380, Applied Biosystems, Foster City, Calif.) and purified by high-performance liquid chromatography and acrylamide gel electrophoresis. Hybridization of this probe to cloned plasmid DNA and DNA obtained from clinical samples was performed either in gel or with nylon membranes after radiolabeling the B19 oligonucleotide probe with phosphorus 32–labeled adenosine triphosphate T4 polynucleotide kinase.[8] The conditions of hybridization for both the dried gels or nylon membranes was at 60° C for 8 to 12 hours in 6× standard saline citrate buffer contain-

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Table I. Nonimmune fetal hydrops cases evaluated

Case	*Gestational age (wk)**	*Fetal hemoglobin (gm/dl)*	*Human parvovirus (DNA)*	*Fetal outcome*
3476	29	14.9	Negative	Live birth
9802	21	12.1	Negative	Fetal death
8217	35	13.5	Positive	Infant death
5653	32	15.2	Negative	Neonatal death
7721	32	9.1	Negative	Neonatal death
2356	22	2.5	Positive	Termination
1121	30	15.0	Negative	Live birth
5183	37	12.6	Positive	Neonatal death
3325	36	17.1	Negative	Neonatal death

*At blood sampling.
†Of percutaneous umbilical blood sample.

ing 10 μg/ml transfer ribonucleic acid and 2×10^{-6} cpm/ml of the B19 oligonucleotide probe.[8] After hybridizations the matrixes were washed in 6× standard saline citrate buffer for 2 to 3 hours at room temperature and 30 minutes at 65° C. Gels and membranes were then autoradiographed with intensifying screens at −70° C for 1 to 3 days.

The primer set for enzymatic amplification of the parvovirus B19 genome by polymerase chain reaction was synthesized and purified in the same way as B19 oligonucleotide probe. The 5′ primer was identical to nucleotides 1667 through 1686 of the parvovirus genome; the 3′ primer was the reverse compliment of nucleotides 1921 through 1940 of the viral genome.[7] The primers for the human genomic positive standard were composed of a pair of oligonucleotides that amplify a 700 base-pair segment previously described in the human actin gene.[9] The conditions for the polymerase chain reaction amplification were optimized for a 100 μl reaction volume with 2.5 units of Taq polymerase (AmpliTaq, Perkin-Elmer Cetus, Norwalk, Conn.). Each reaction mixture contained 50 mmol/L potassium chloride, 10 mmol/L Tris pH 8.3, 2.5 mmol/L magnesium chloride, 1.0 mmol/L deoxynucleoside triphosphates, 40 pmol/L each primer, 4 mmol/L spermidine hydrochloric acid, and various amounts of template DNA as noted in results. Each reaction mixture was overlayed with mineral oil; after an initial incubation at 97° C for 4 minutes, 30 cycles of amplification were carried out, each consisting of 1 minute at 94° C and 3 minutes at 65° C. After the amplification 10 μl aliquots of the reaction mixture were either subjected to electrophoresis in 2% agarose gels for 1 hour at 60 V or spotted onto nylon membranes with a dot blot device. Nonisotopic detection of the amplification product was done by staining gels with ethidium bromide for 15 minutes; the gels were then visualized and photographed with ultraviolet light.

The known positive standard template DNA source was the plasmid pYT104C (a gift from P. Tattersall) containing the entire coding sequence of a human parvovirus B19 isolate.[7] Before hybridizations and polymerase chain reaction amplifications the plasmid DNA was linearized by *Sal* I digestion.

Clinical samples consisted of 1.5 ml of amniotic fluid or 0.25 ml of fetal cord blood obtained antenatally by ultrasonographically guided amniocentesis or percutaneous umbilical blood sampling from gestations initially seen at various gestational ages with hydrops fetalis. Samples of maternal peripheral blood were also obtained at initial evaluation. The basic clinical aspects of the gestations evaluated are summarized in Table I. Blood and amniotic fluid samples were prepared for dot-blotting or polymerase chain reaction by a rapid freeze-thaw followed by centrifugation at 12,000 rpm in a microfuge for 5 minutes to obtain a supernatant free of cell debris. In both the dot blot and polymerase chain reaction experiments 20 μl of supernatant was used without further treatment. Approval for use of the test on material from human subjects was obtained from the institutional research committee.

Results

The sensitivity and specificity of the B19 oligonucleotide probe and the optimal conditions for hybridization were determined by dot blots with serial dilutions of DNA isolated from plasmid pYT104C suspended in buffer. The quantity of DNA spotted onto the nylon membrane for this determination ranged from 10 pg to 1 μg. In addition, 0.1 μg of plasmid DNA was added to 0.5 ml lysed whole blood from an uninfected individual and spotted onto the nylon membrane. An additional control of 0.5 ml lysed whole blood without pYT104C DNA was also used. The results of these hybridizations are shown in Fig. 1. This experiment demonstrated that, under our conditions, the B19 oligonucleotide probe detected as little as 10 pg of the parvovirus DNA, equivalent to about 2×10^5 viral particles. Parvovirus DNA was also detected in the presence of whole blood, although the signal strength was slightly less intense than that seen with an equivalent quantity of target DNA suspended in buffer. Thus

in the presence of whole blood the lower limit of sensitivity was about fivefold less than the sensitivity in buffer, or about 10^6 viral particles. No hybridization signal was detected at the spot containing only lysed whole blood, indicating absence of detectable nonspecific hybridization to human DNA.

We next used the primers B19A/B with the linearized whole plasmid DNA for polymerase chain reaction amplifications. To optimize the reaction, various parameters of thermal cycling were tried. In these experiments we used a 1 μg quantity of pYT104C DNA as the target for amplifications. Negative controls consisted of 1 μg of human DNA, a 30 μl sample of lysed whole blood (both obtained from a noninfected person), or an aliquot of sterile buffer. Gel electrophoresis of the amplification reaction products revealed that after an initial denaturation step at 95° C for 1 minute the 274-base-pair product was best amplified with a single temperature for annealing and extension. The negative controls did not yield any amplification product. To document that the amplification product was derived from the parvovirus genome the gels were hybridized to radiolabeled B19 oligonucleotide probe. The autoradiographs from this hybridization demonstrated that the DNA fragment amplified was specific for the segment of the parvovirus genome detected by the parvovirus-specific probe. We next determined the minimum quantity of parvovirus DNA necessary for amplification. Setting the number of amplification cycles at 30, with 1 μg of each primer 1 μl aliquots of serial dilutions of pYT104C plasmid DNA ranging from 100 pg to 0.01 pg were subjected to amplification. After this 50 μl quantities of the reaction products were electrophoresed for 1 hour, stained with ethidium bromide, and photographed. As shown in Fig. 2 these experiments demonstrated that as little as 1 pg or 2×10^4 viral particles could be amplified and detected under our conditions. When this experiment was repeated with the same amounts of pYT104C DNA suspended in whole blood lysate a reduction in sensitivity was again found. Thus under these conditions our lower limit of detection was 5 pg of pYT104C DNA, equivalent to 1×10^5 viral particles.

After these preliminary studies clinical samples obtained at prenatal evaluation of fetal hydrops were assayed by our polymerase chain reaction method with the optimized parameters developed in our preliminary studies. The results of these amplifications are shown in Fig. 3. With the method described we were able to detect the presence of parvovirus B19 in fetal blood samples in three of nine cases in which nonimmune hydrops was detected by ultrasonography. However, only in case 2356 was the virus also detected in amniotic fluid. In addition, as determined by visual inspection of the ethidium bromide–stained gel, the amount of virus in the fluid was much less than in the fetal blood in that the 274-base-pair amplification product was barely detectable. In all cases the success of polymerase chain reaction amplification was established by the presence of the 700-base pair actin sequence on the gels. In no cases was parvovirus B19 detectable in the samples of maternal blood obtained at the time of fetal sampling.

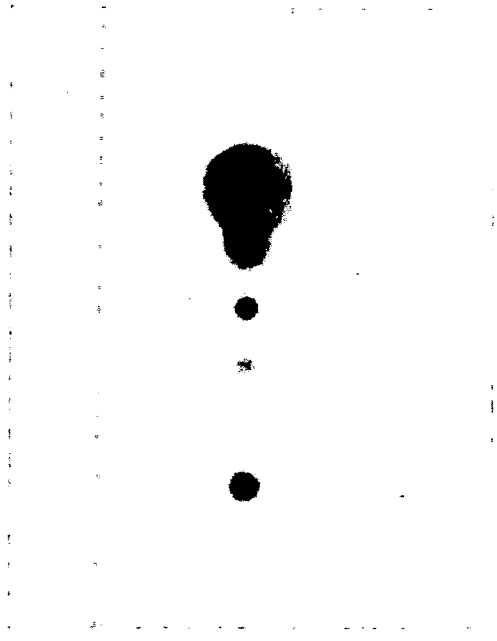


Fig. 1. Hybridization of B19 oligonucleotide probe to dilutions of parvovirus DNA. The range of concentration is from 1 μg of DNA in the first dot to 10 pg in the fifth dot. Positions 2 and 6 contain equivalent amounts of parvovirus DNA (0.1 μg) mixed with sterile buffer and whole blood lysate, respectively. Position 7 contains a negative control of whole blood lysate alone.

In all of the positive cases follow-up polymerase chain reaction analysis was done on fetal samples. In case 8217 the clinical manifestations of hydrops resolved and the patient was delivered of a male infant without any apparent sequelae. Test results for a cord blood sample obtained 4 weeks after the initial sample were still positive for parvovirus B19 in the subsequent polymerase chain reaction analysis. Notably, in this fetus the hydrops resolved within 4 weeks of our evaluation, and the pregnancy resulted in a live-born male infant without apparent problems. This infant died at home 53 days after delivery under circumstances presumed to be consistent with sudden infant death syndrome. An autopsy was not obtained on this infant precluding further analysis.

In case 2356 the hydrops persisted and worsened over a 7-day period. At 25 weeks' gestation ultrasonography revealed massive pericardial effusion, ascites, and no cardiac motion in the fetus. After documen-

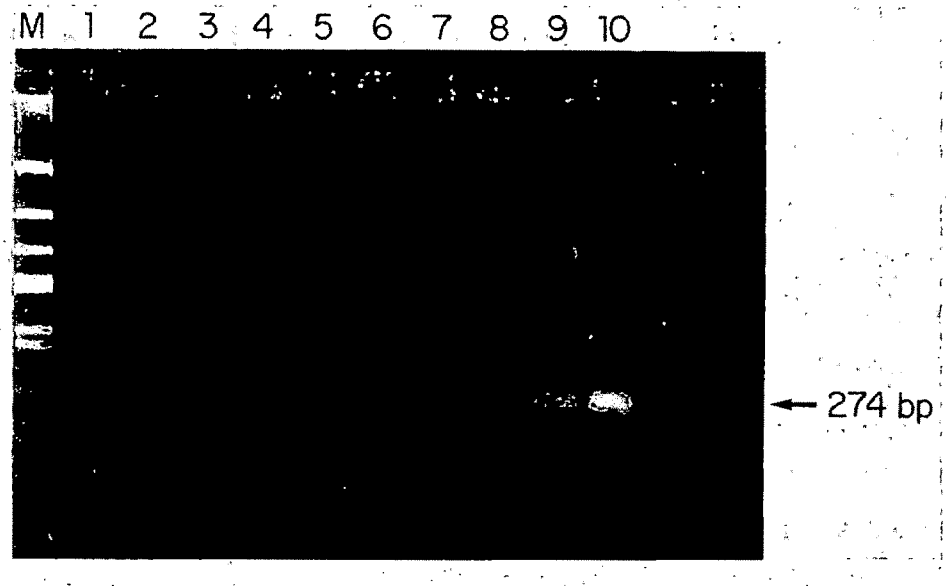


Fig. 2. Gel electrophoresis of polymerase chain reactions with various amounts of parvovirus B19 DNA with whole blood lysate. *Lanes 1, 2,* and *3* are negative controls with sterile buffer, 1 μg purified human DNA, and whole blood lysate as the amplification template. *Lanes 4* through *9* are amplifications with 0.1 pg, 1 pg, 5 pg, 50 pg, 100 pg, and 500 pg quantities of parvovirus DNA in whole blood lysate. *Lane 10* is a positive control of 500 pg of parvovirus DNA in sterile buffer. Fragment size is indicated in base pairs *(bp)*.

tation of an intrauterine fetal death the pregnancy was terminated. No autopsy was performed on this fetus; however, after delivery a portion of the placenta and fetal blood from cardiac aspiration was obtained, and test results for both were positive for parvovirus B19 with our assay method.

Case 5181 was delivered 36 hours after fetal blood sampling. The infant was delivered with marked hypotension and was admitted to the neonatal intensive care nursery, where he died 12 hours after delivery. Blood samples obtained in the nursery were sent for viral cultures, which were positive for parvovirus B19. In this case an autopsy revealed a 3280 gm male infant with generalized hydrops and edema. Hepatosplenomegaly was noted, along with an enlarged heart. Histopathologic examination of the heart demonstrated generalized inflammatory changes. The liver demonstrated perivascular mononuclear infiltrates. These findings were believed to be consistent with those reported in other cases of fetal infection with several viruses, including parvovirus B19.[5] Test results for a portion of fetal cardiac muscle and spleen tissue obtained at autopsy were positive for parvovirus B19 with the polymerase chain reaction assay.

The maternal histories obtained in the three positive cases were notable for several features. In case 8217 the mother gave a history of recent travel to Mexico to visit family. During that visit she came in contact with several children who had symptoms resembling "influenza." However, she never experienced any symptoms. In case 2356 the mother gave a history of "flu" with fever approximately 4 weeks before evaluation. Case 5181 was associated with maternal exposure to a child with nausea, vomiting, fever, and a "red face" in her home about 5 weeks before evaluation. Parvovirus-specific immunoglobulin studies were not obtained in any of these cases.

Comment

The polymerase chain reaction is a rapid and relatively simple molecular genetic technique that enzymatically amplifies exceptionally small amounts of a specific DNA present in clinical samples. Since the first description of this technology the polymerase chain reaction method has been applied to clinical specimens for diagnosis of a variety of neoplastic, genetic, and infectious diseases.[10] Indeed, at the same time the studies reported here were underway another group of investigators also reported on the use of polymerase chain reaction to detect the presence of human parvovirus B16 in formalin-fixed tissues obtained at autopsy from a case of an intrauterine fetal death.[11] In our study we used the polymerase chain reaction technique to amplify a different portion of the viral genome and demonstrated that the sensitivity of this method exceeds that which can be obtained with more traditional approaches, such as dot-blots. Importantly, both studies show that the detection of the viral genome was specific in that known parvovirus-negative samples did not yield amplification product. However, as has been

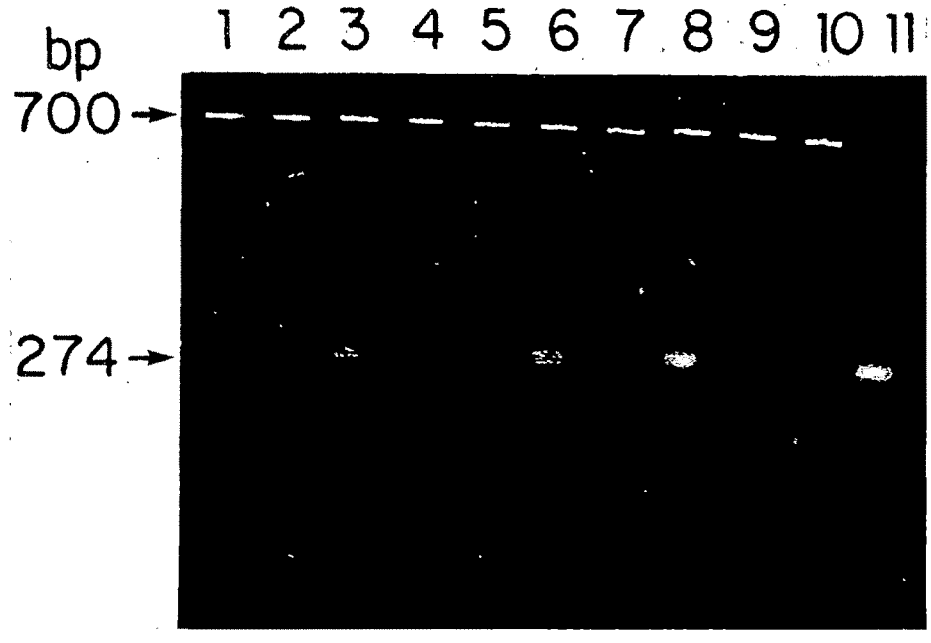


Fig. 3. Gel electrophoresis of the amplification products obtained from fetal blood samples in the nine cases initially seen with hydrops fetalis are seen in *lanes 1* through *9*. *Lane 10* is a negative control, and lane 11 is a positive control. The 274-base-pair *(bp)* fragment is the parvovirus amplification product, and the 700-base-pair fragment corresponds to the human actin standard.

noted by others, contamination of samples with exogenous target DNA template can be a potential problem in polymerase chain reaction–based assays.[10] Therefore appropriate measures must be taken to avoid this problem, and negative controls should always be used in amplification protocols.[12] Moreover, analysis of paired maternal samples will preclude the possibility of false-positive results resulting from cross-contamination with viral particles in maternal blood.

We also found that the sensitivity of the assay decreased in the presence of lysed whole blood. This is not entirely unexpected and is most likely the result of inhibition of the *Taq* polymerase enzyme by heme present in the sample. Alternatively, it could also be caused by some nonspecific binding of the oligonucleotide primer sequences to human genomic DNA possessing some homologic sequence because the effect was also noted in the dot blots. In the polymerase chain reaction amplification process nonspecific binding would result in a reduction of primer/template ratio, which would in turn affect amplification efficiency.[10] However, the sensitivity of the assay, even in the presence of blood, is at the level of about 100,000 viral particles. Moreover, the use of radioactive nucleotides in the polymerase chain reaction would result in even greater sensitivity, as would increases in the number of amplification cycles, without affecting specificity.

In our study we have demonstrated that parvovirus B19 DNA can be detected in amniotic fluid and fetal blood samples obtained in a prenatal diagnostic setting with enzymatic DNA amplification. This technique represents an extremely sensitive detection method that is especially useful when small sample size limits diagnostic techniques. It is also more rapid than viral culture for diagnosis, requiring only a few hours to perform. Morever, in applying this technique to a group of clinic patients we found that some gestations with signs of nonimmune fetal hydrops have definitive evidence of parvovirus B19 infection.

The basic pathogenesis of fetal hydrops is thought to be that of cardiovascular failure as precipitated by anemia, myocardial dysfunction, vascular shunts, and other alterations in cardiovascular dynamics.[4] In the case of parvovirus B19 infection, anemia or direct cytopathic effects on fetal organs, including the myocardium, have both been postulated to be responsible for the fetal hydrops.[13] In the cases reported here anemia was noted in the fetal blood samples, a finding that is consistent with other reports of fetal infection with parvovirus B19 but is not an invariable finding in all cases.[13] On the basis of the limited number of cases in this series we cannot speculate on the true incidence of anemia associated with human parvovirus B19 in fetuses initially seen with nonimmune hydrops. Neither can we be certain that the pathogenesis of the hydrops was in fact caused only by the anemia. The presence of detectable parvovirus in the myocardium of one case combined with relatively mild anemia in another suggests that more than erythroid cell destruction may contribute to the fetal hydrops; this has also been suggested

in other studies of fetal parvovirus B19 infections.[13] Most important, however, the variable outcomes of the pregnancies seen in our study indicate that many additional questions regarding the association of human parvovirus B19 infection and nonimmune hydrops remain to be answered. In our series we noted a live-born infant without obvious sequelae after an in utero infection. Thus our case of a documented fetal viremia and hydrops that spontaneously resolved adds to those cases reported by others of apparently normal outcomes in some fetuses after in utero parvovirus B19 infections.[13] However, in our case a sudden infant death without a definitive cause of death is disturbing and might be related to early and apparently persistent chronic infection. This case emphasizes that much is yet to be learned about the pathologic make-up of parvovirus infection and its long-term effects on fetuses that have viremia in utero. In sum, on the basis of the data presented here we believe that the simplicity of this diagnostic method presents clinicians with an important tool for evaluation of nonimmune fetal hydrops. When applied to larger clinical studies this method will be an important tool in understanding the natural history of parvovirus B19 infection occurring in pregnancy.

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Angina and normal coronary arteries in women: Gynecologic findings

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OBJECTIVES: Our aim was to evaluate the hypotheses that women with severe angina and normal coronary arteries (syndrome X) have an increased incidence of hysterectomy and show a positive cardiac response to 17β-estradiol replacement therapy.

STUDY DESIGN: The gynecologic histories of 30 women with syndrome X were determined. Anginal and 17β-estradiol insufficiency symptoms were recorded daily for 1 month. Subsequently, 20 of the women underwent hyperemic response testing before and after 2 months of estrogen replacement therapy. Hyperemic response results were compared with those of 12 asymptomatic post-menopausal women not receiving estrogen replacement therapy. Symptoms were recorded daily during estrogen replacement therapy.

RESULTS: A total of 18 women (60%) underwent hysterectomy. All were experiencing hot flushes. Hyperemic response was diminished in women with syndrome X compared with controls. Hyperemic response increased and anginal symptoms decreased during estrogen replacement therapy.

CONCLUSION: The incidence of hysterectomy was increased and estrogen replacement therapy alleviated cardiac symptoms and enhanced hyperemic response in a group of women with syndrome X. (Am J Obstet Gynecol 1992;167:467-72.)

Key words: Angina, estrogen, hysterectomy, vasodilatory reserve, syndrome X

Women with severe angina, exercise electrocardiogram changes suggesting ischemia, and normal coronary arteries (referred to as syndrome X or microvascular angina) present a challenging medical problem. These patients have an excellent prognosis with regard to myocardial infarction and cardiovascular mortality.[1] Nevertheless, existing cardiovascular medications are frequently ineffective in relieving pain, and follow-up studies indicate that many patients have significant limitation of daily activities because of pain.[2] Patients with this condition have shown a diminished hyperemic response, suggesting the chest pain to be a manifestation of a more widespread vascular disorder.[3]

One of the earliest observations about angina in women was made by Sir William Osler,[4] who in 1892 pointed out that anginal pain without coronary artery disease was more common in women than in men, carried a good prognosis, and was characterized by discomfort that was often long-lasting. LaCroix et al.,[5] in a recent national study reported by the Epidemiology Branch of the National Institutes of Health, found among white women aged 25 to 54 years that diagnosed angina occurs twice as often as it does among age-matched white men. In spite of the clinical diagnosis of angina pectoris, >50% of women undergoing coronary angiography have normal coronary arteries compared to <10% of men.[6] For these women an etiologic explanation for the chest pain remains elusive and a variety of noncardiac causes have been postulated.

Angina pectoris in women is frequently associated with other conditions of vascular disorder. These include hot flushes,[7] Raynaud's phenomenon,[8-10] and migraine.[11] In the study of LaCroix et al. 20.5% of the women with angina also experienced severe dyspnea, "... requiring the subject to stop for breath when walking on level ground."[5] In contrast, only 4.5% of the men in the same age group experienced severe dyspnea. Kronenberg[7] reported that 16% of 438 perimenopausal and postmenopausal women experienced significant chest pressure and 28% experienced cranial pressure when hot flushes occurred. Among women with Raynaud's phenomenon, an increased incidence of variant angina and migraine has been reported.[9, 10]

The association of angina and coronary artery disease with loss of ovarian function has been described in many articles in the medical literature. Wuest et al.[12] reported that women who underwent bilateral oopho-

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rectomy and did not receive hormone replacement therapy showed an increased prevalence of severe atherosclerotic disease at autopsy, and Robinson et al.[13] found that castrated women showed a higher incidence of angina as compared with that of controls who had not had surgery. Although myocardial infarction and cardiovascular disease are most clearly associated with bilateral salpingo-oophorectomy,[14] elevated risks of cardiovascular disease have been reported even after simple hysterectomy,[15] and loss of ovarian hormone production after simple hysterectomy has been suggested as an etiologic factor.[16]

The possibility that ovarian insufficiency might be associated with syndrome X in women led to a gynecologic endocrine evaluation of 30 patients with this condition. The patients were seen at the Royal Brompton and National Heart Hospital in London, where their gynecologic histories and signs and symptoms of ovarian insufficiency were evaluated. Twenty of these women subsequently participated in a pilot study to determine whether 17β-estradiol treatment would have a positive effect on cardiac symptoms and measures of cardiovascular function.

Patients and methods

Thirty consecutive female patients previously diagnosed with syndrome X were recruited for this study. Before our investigations all of the women had been evaluated for chest pain by their family physicians and at least two consulting cardiologists. These physicians had concurred that the nature of the women's pain fit the criteria for a diagnosis of angina pectoris. The women were seen in the Cardiac Medicine Clinic of the Royal Brompton and National Heart Hospital in London. They were interviewed by a research team consisting of a cardiologist (DL or GR) and a gynecologist (PS). This interview further confirmed the presence of angina-type chest pain. In fact, all the women had a history of severe angina, a positive exercise test (ST segment depression >1 mm during treadmill testing), and normal coronary arteries at the time of coronary catheterization. The women were receiving a variety of cardiac medications including coronary vasodilators, calcium channel–blocking agents, β-adrenergic–blocking agents, and cardiac glycosides. The cardiac history was reviewed, and as complete an obstetric and gynecologic history as possible was elicited. A blood sample was drawn for determination of serum estradiol and follicle-stimulating hormone (FSH) concentrations. The control group comprised 11 women without chest pain or hot flushes (age 52.6 ± 4.5 years, mean ± SD). The women in the control group were not receiving hormone replacement therapy although all were postmenopausal, showing estradiol concentrations <25 pg/ml and FSH levels >40 mIU/ml. The women in the control group were not taking any cardiac medications. Patients with a history of hypertension were excluded from both groups.

After informed consent was obtained, all cardiac medications were discontinued for at least 1 week except for sublingual glyceryl trinitrates. Glyceryl trinitrate treatment was discontinued for a minimum of 24 hours before the first study visit. The subjects in both groups were not allowed to smoke cigarettes or drink tea or coffee within 4 hours of the study. Ten of the women with syndrome X either were taking or recently had been taking hormone replacement therapy. These medications were all discontinued for at least 6 weeks before the initial hyperemic response was measured.

The subjects reclined in a semirecumbent position on a couch, and blood pressure was recorded from the left arm. Hyperemic response was measured with a laser Doppler probe applied to the pulp of the right middle finger. The room temperature was maintained at 70° F. After a period of stabilization, arterial flow in the fingertip was determined for a 2-minute period. Limb blood flow was then occluded for 5 minutes with a sphygmomanometer inflated to 200 mm Hg. The laser Doppler probe continued to monitor flow through the period of occlusion and for 3 minutes after release of the cuff pressure.

All of the women maintained a daily diary for 1 month, during which they recorded the frequency and intensity of chest pain, sleep disturbance, headache, anxiety reactions, and depression. A rating scale ranging from 0 (no symptoms) to 4 (severe symptoms) was used. A minimum of a two-level change in a symptom (e.g., from 4 to 2) defined a "significant change."

At the follow-up visit, 20 of the women diagnosed with syndrome X agreed to participate in a 2-month trial in which they wore 17β-estradiol skin patches (Estraderm TTS) delivering 50 μg/day for the first month and 100 μg/day for a second month. The estradiol patches were changed twice weekly and were applied to the buttocks according to the usual prescription guidelines. The women agreed to discontinue cardiac medications except for sublingual nitrates. The diaries were maintained throughout this period, and the hyperemic response test was repeated at the end of the trial. The 10 women initially evaluated who were not included in the estradiol trial were those who were not willing to return for repeat visits because of the inconvenience of coming to the clinic in London, did not wish to receive estrogen treatment, or had return appointments after recruitment for the estrogen treatment study was completed.

Findings and results

Hormonal and nonanginal vascular findings are summarized in Fig. 1. The mean age of the women was 52 years (range 46 to 63, SD ± 6). Twelve of the women had experienced a natural menopause at a mean ± SD

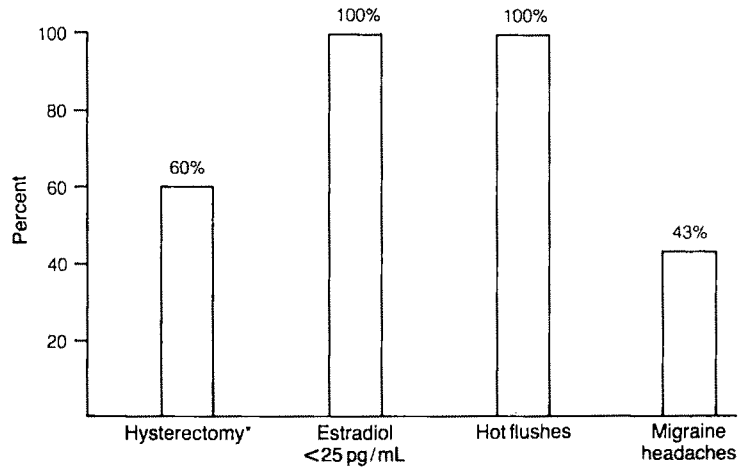


Fig. 1. Hormone and vascular findings in 30 women with syndrome X. Mean ± SD age of women was 52 ± 6 years. Average ± SD time from hysterectomy to onset of cardiac symptoms was 7.7 ± 5.7 years.

age of 49.5 ± 4 years (range 47 to 55). Eighteen of the 30 women underwent hysterectomy at an average age of 41 years. Sixteen of the 18 hysterectomies were abdominal procedures, and two women had vaginal hysterectomy for uterine prolapse and urinary incontinence. We were not able to determine the gynecologic diagnoses in many of the women as they themselves were unsure. However, it did appear that essentially all of the procedures were necessary, with the most frequent indications being uterine fibromyomas or excessive bleeding or both. Nine of the 18 women had ovaries removed at the time of surgery. Seventeen of the 30 women had received hormone replacement therapy at some time. However, only 10 of the women were taking hormone replacement therapy when first seen in this study. Nine of the 10 women using hormones had undergone hysterectomy, and eight of these women had bilateral oophorectomy. Seven women had discontinued hormone replacement therapy, including four who had been given estrogen plus progestin. These four women stopped hormone replacement therapy because of intolerable chest pain that developed during the time they were taking the progestin.

Angina developed at a mean ± SD duration of 7.7 ± 5.7 years after hysterectomy.

All the women, including those taking hormone replacement therapy, were experiencing hot flushes. In five of these women the flushes were mild (level 2), but 25 women rated the flushes as severe (level 4) in the monthly diaries. Sleep was disrupted in all of the women, usually because of awakening with hot flushes. All the women reported that at least some of their hot flushes were accompanied by chest pressure or pain. Thirteen of the 30 women had migraine headaches.

Serum estradiol and FSH concentrations were <25 pg/ml and >40 mIU/ml, respectively, in all of the women.

Hyperemic response results before 17β-estradiol treatment in the syndrome X women are compared with those of an age-matched, pain-free control group in Fig. 2. The ratio of postocclusion blood flow level to the preocclusion level was calculated at 20-second intervals for 2 minutes. It can be seen that the women with syndrome X do not show any response above baseline (i.e., they do not appear to have a vasodilator reserve), whereas the control group shows a significant response over the entire 2 minutes. Calculation of the ratio of the maximum response level to the baseline flow level (MBR) gave a value of 1.08 for those with syndrome X and 2.3 for the control group.

After estradiol treatment for 2 months, the MBR of the women with syndrome X was 2.07. The posttreatment MBR is compared with pretreatment and control group findings in Fig. 3. Response levels were almost doubled after estradiol treatment and approached the levels of the control group without angina.

Nineteen of the 20 women reported marked improvement (a minimum of a two-level decrease in the anginal symptom rating) or complete disappearance of chest pain. Cessation of hot flushes correlated with decrease or disappearance of chest pain. All of the women reported a decrease in the use of glyceryl trinitate tablets. Unfortunately, the diaries did not include quantification of glyceryl trinitate usage, and therefore we are not able to report the decline in usage with precision.

Comment

These findings suggest an association between hysterectomy, ovarian insufficiency, and the development of syndrome X. The average age of the patients studied was 52; in Great Britain, 8% to 12% of women in this age group have undergone hysterectomy with or with-

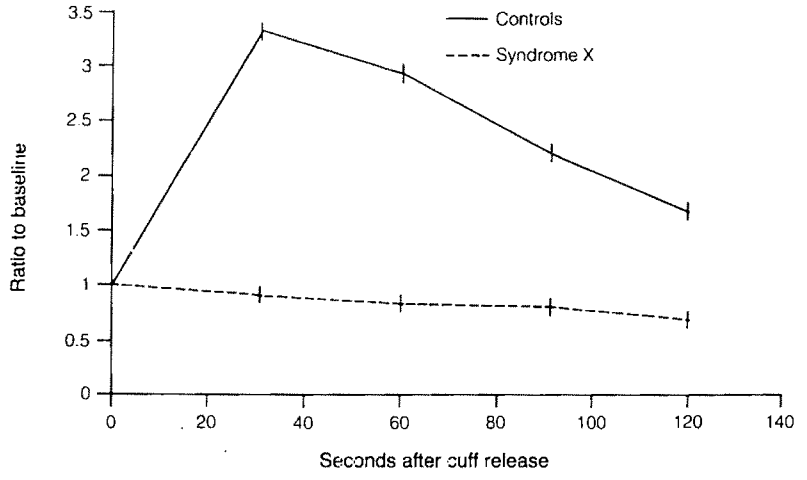


Fig. 2. Hyperemic response of 20 women with syndrome X compared with that of 12 control subjects. (See text for details of test.)

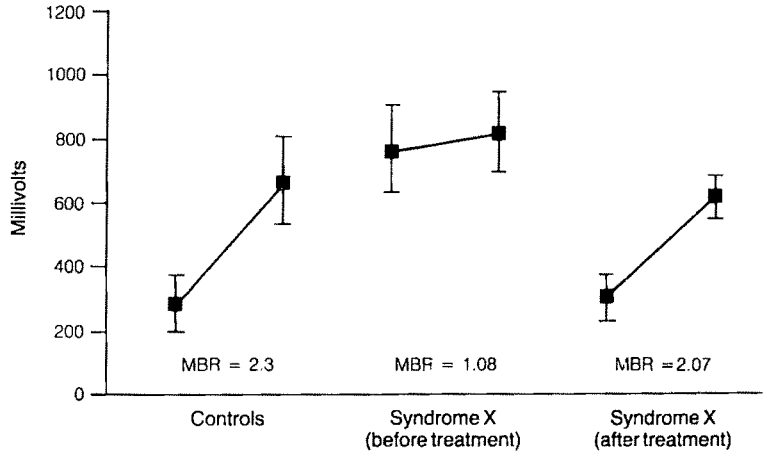


Fig. 3. MBR of hyperemic response in women with syndrome X before and after treatment with 17β-estradiol compared with that of control subjects.

out removal of the ovaries.[17] In the current series 60% had undergone hysterectomy. All of the hysterectomies appear to have been indicated procedures.

Although the evidence in the medical literature relating hysterectomy to the development of coronary artery disease is more clearly associated with bilateral salpingo-oophorectomy,[14] studies indicate that simple hysterectomy is also reported to incur an elevated cardiovascular risk. As noted, the study of Siddle et al.[16] implies that the loss of ovarian hormone production with simple hysterectomy may be comparable to that occurring when the ovaries are removed. In the latter instance hormone replacement therapy is more likely to be initiated soon after the surgery. Unfortunately, when the ovaries are retained, it can be mistakenly assumed that normal ovarian function always ensues and there is no need for hormone replacement therapy. Moreover, most patients are lost to follow-up after hysterectomy, since neither gynecologists nor patients believe that continuing gynecologic care is necessary. In the current series only three women returned to their gynecologists for continuing follow-up after their scheduled postoperative visits. Nonetheless, most of the women with retained ovaries described symptoms of ovarian insufficiency to their primary care physicians. However, since their ovaries had not been removed, their physicians did not attribute the symptoms to ovarian insufficiency, and only one woman received hormone replacement therapy. Cardiac symptoms developed within 7 years of the hysterectomy.

Chest pain is not considered a reliable indicator of angina pectoris in women.[18] The cardiologists involved in the characterization of the chest pain in the women in our series determined to the best of their abilities that the pain was of cardiac origin, and the positive results of exercise tests would seem to confirm the impression of ischemic disease. The finding of a decreased vasodilatory reserve capacity in the syndrome X women compared with the age-matched control women suggests the problem to be due to altered cor-

onary hemodynamics in association with pathophysiologic changes other than atherosclerotic plaque formation.

All the women showed symptoms of estradiol deficiency. Twenty-five of 30 were experiencing >20 hot flushes each day. Chest pain frequently accompanied the hot flushes. Further evidence of a more widespread vascular disturbance was seen among 13 (43%) of the women who also experienced migraine headaches (Fig. 1).

The pretreatment hyperemic response results in the syndrome X women indicate diminished vasodilator reserve capacity. This finding confirms the observations of others who have concluded that reduced hyperemic response in syndrome X indicates the anginal symptoms to be a manifestation of a widespread vascular disorder.[3] The hyperemic response in the forearm to the release of arterial occlusion has been extensively studied. Sudden increase in blood flow in an artery is thought to stimulate the release of endothelial-derived relaxing factor (EDRF), and the hyperemic response is thought to reflect vasorelaxation induced by EDRF acting on the vascular smooth muscle.[3] Our finding of increased hyperemic response after 17β-estradiol treatment is consistent with those of others, including Williams et al.,[19] who reported that coronary vascular response to acetylcholine stimulation (another EDRF-mediated vasodilator response) in ovariectomized monkeys is increased after 17β-estradiol implants.

Hemodynamic changes reported after treatment with 17β-estradiol include restoration of vasomotor control, increased cardiac output, and decreased vascular resistance.[20] Increased hyperemic response correlated in our study with an increased vasodilator reserve as the syndrome X patients showed significant improvement in cardiac symptoms.

Estrogen replacement therapy in postmenopausal women has been shown to reduce cardiovascular morbidity and mortality.[21] Although effects on lipid metabolism appear to contribute significantly to this cardioprotection, the hemodynamic effects of 17β-estradiol also appear to be of significance. The current study shows an association between signs and symptoms of ovarian insufficiency and disturbed vascular function in women with syndrome X. The part of the study assessing the effects of 17β-estradiol treatment on cardiac symptoms and hyperemic response should be considered a pilot investigation into a potential mechanism through which estrogen treatment provides cardioprotection.

This study indicates that estradiol deficiency may induce pathophysiologic changes contributing to anginal symptoms in women diagnosed with syndrome X. The high prevalence of hysterectomy and the inadequacy of hormone replacement therapy observed in this study suggest the need for careful gynecologic endocrine evaluation of women presenting with this syndrome. A double-blind, placebo-controlled study of the effects of 17β-estradiol on cardiovascular parameters in women with syndrome X should determine which hormone effects account for the improvements seen thus far.

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Modulation of cell-to-cell coupling between myometrial cells of the human uterus during pregnancy

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OBJECTIVE: The purpose of this study was to investigate changes in cell-to-cell coupling of human myometrium during pregnancy to assess the presence and permeability of gap junctions.
STUDY DESIGN: To evaluate the coupling, input resistance was measured and intercellular spread of Lucifer yellow was observed with microelectrode techniques in intact myometrial preparations from four nonpregnant women, 13 women not in labor, and three women in labor. Octanol, isoproterenol, and dibutyryl adenosine 3′,5′-cyclic monophosphate were applied to the preparations to assess their effects on cell-to-cell coupling.
RESULTS: Input resistance of myometrial cells was decreased ($p < 0.001$) and intercellular spread of Lucifer yellow was increased during pregnancy. Octanol, isoproterenol, and dibutyryl adenosine 3′,5′-cyclic monophosphate rapidly and reversibly increased input resistance ($p < 0.001$ for all these agents) and blocked Lucifer yellow spread in tissues from pregnant patients.
CONCLUSIONS: Cell-to-cell coupling between human myometrial cells is spontaneously improved during pregnancy because of the presence of gap junctions. The coupling is rapidly and reversibly decreased by octanol, isoproterenol, and dibutyryl adenosine 3′,5′-cyclic monophosphate as a result of decreased permeability of gap junctions. These two methods of modulation of gap junctions are suggested to be major mechanisms for control of myometrial contractile activity in the human uterus during pregnancy. (Am J Obstet Gynecol 1992;167:472-80.)

Key words: Human myometrium, cell-to-cell coupling, β-adrenergic receptor agents, adenosine 3′,5′-cyclic monophosphate, gap junction

Gap junctions increase between smooth muscle cells of myometrium during labor and parturition in rats, humans, and other species.[1-5] These specialized cell-to-cell contacts are thought to provide intercellular pathways that allow the synchronization of electric and metabolic activities. Therefore the appearance of gap junctions between smooth muscle cells is thought to result in coordinated muscle activity due to better coupling between cells.

Previous morphologic studies of the human uterus have shown that gap junctions were present between myometrial cells in high numbers in term women with frequent uterine contractions or premature labor,[5] whereas they were present in very low frequency in women with infrequent uterine contractions and absent in most tissues from nonpregnant women.[6] In the myometrium of other mammalian species indirect studies of cell-to-cell coupling have shown that the muscle cells become better coupled when the gap junctions are elevated during labor.[7-9] However, there are no functional studies on the human uterus that have adequately described the change in the coupling between myometrial cells either during pregnancy or in the nonpregnant state. Recent studies have used microelectrode techniques to evaluate the coupling between myometrial cells by measuring input resistance and metabolic coupling (fluorescent dye injection).[10,11] A decrease in input resistance and an increase in dye spread between rat and guinea pig myometrial cells suggested that the coupling between muscle cells improved when gap junctions were increased in the myometrium at term.[10-12]

The purpose of this study was to use intracellular microelectrode techniques to functionally assess cell-to-cell coupling between cells of intact myometrial preparations from pregnant and nonpregnant women. The present study demonstrates that the coupling between human myometrial cells is dramatically improved during pregnancy because of the appearance of gap junctions. Furthermore, this study shows that octanol and agents that elevate intracellular adenosine 3′,5′-cyclic monophosphate concentration will rapidly and reversibly lower the coupling presumably through closure of the gap junctions.

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Material and methods

Tissues were collected for this study from two groups of women at term pregnancy. Women in one group underwent elective cesarean section ("before labor"), and women of the other group sustained cesarean section for cephalopelvic disproportion when the cervical dilatation was ≥5 cm with contraction frequency being at least 3 per 10 minutes ("during labor"). Small segments (approximately 2 × 2 × 0.5 cm) of pregnant myometrium were excised from the superior aspect of the lower uterine incision. Similar sizes of myometrial segments from the fundus of the nonpregnant uterus were obtained from women who experienced hysterectomy with no obvious pathologic condition. This study was approved by the institutional review board. Informed consent to remove the tissue and participate in the study was obtained from each patient.

Tissue preparation. Strips of tissue were immediately placed in physiologic saline solution and stored at 4° C. All experiments were started <3 hours after obtaining the tissues. Each tissue was placed in an organ bath containing well-aerated (95% oxygen and 5% carbon dioxide) Krebs solution and pinned at its resting length. The connective tissue surrounding the tissue was carefully removed, and a thin preparation of the myometrium was made with fine curved forceps under a dissecting microscope. The myometrial preparation, approximately 1.5 × 1.5 × 0.1 cm, was cut free and pinned at its resting length to a rubber ring cemented on the bottom of a 35 mm Petri dish with a glass coverslip glued under a hole in the center. The dish was filled with Tyrode's solution (composition 135 mmol/L sodium chloride, 5 mmol/L potassium chloride, 1 mmol/L magnesium chloride, 1.8 mmol/L calcium chloride, 5.6 mmol/L glucose, 10 mmol/L Hepes, 0.05% gelatin) that was adjusted to pH 7.4 and warmed to 37° C. The myometrial preparation was viewed under an inverted microscope (Ernst Leitz, Wetzlar, Germany) equipped with a heating stage (37° C) and phase-contrast optics.

Electrophysiology. Electrodes were pulled from 1.2 mm outer diameter capillary tubes (World Precision Instrument, New Haven, Conn.) with a horizontal micropipette puller (Sutter Instruments, San Francisco). A microelectrode filled with 3 mol/L potassium chloride (40 to 100 MΩ) was connected to an amplifier (World Precision Instrument), and electrical currents were injected to the electrode by a stimulator (Grass Instrument, Quincy, Mass.). Signals were monitored on a two-channel digital storage oscilloscope (Nicolet, Madison, Wis.). The total resistance of an electrode impaled in a cell is determined by the sum of the electrode resistance and the resistance to current flow out of the cell (the cell input resistance). Input resistance

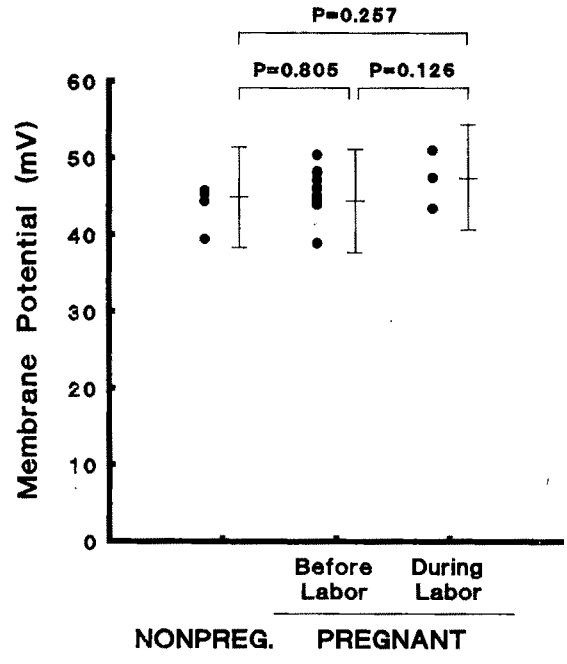


Fig. 1. Membrane potentials (representing intracellular negative values) measured in smooth muscle cells of myometrial preparations from women. *Closed circles,* Average value from impaled cells of preparations obtained from same women (four nonpregnant, 13 before labor, and three during labor); *bars,* mean values ± SEM obtained by combining all values from impaled cells of each group. *p* Values are analyzed by comparing values of membrane potential from impaled cells between groups.

reflects the combined resistance of the junctional membrane, the nonjunctional membrane, and the cytoplasm. Nonjunctional membrane is extremely high (nonjunctional membrane > > junctional membrane), and cytoplasm is nearly 0 Ω. Because changes in both nonjunctional membrane and cytoplasm under conditions of these experiments are thought to be very small, any alteration in cell coupling resistance is indicated by a modification in input resistance. During impalement of the bridge-balanced electrode into a cell, input resistance was determined from the hyperpolarization of electrical potential elicited by an inward current pulse (0.5 nA, 200 msec/sec) from the stimulator. The values of membrane potential and input resistance were accepted if the electrode potential and resistance returned to baseline after removal of the electrode from the cell. In the text and figures, values of membrane potential are shown as absolute values without indicating their negative sign.

Fluorescent dye injection. The injection of Lucifer yellow (Sigma Chemical Co., St. Louis) was used to examine intercellular diffusion capabilities. A 3% solution of Lucifer yellow (Lucifer yellow dissolved in 1% lithium chloride buffered to pH 7.6 with 10 mmol/L Hepes) was back-filled into the microelectrodes (40 to

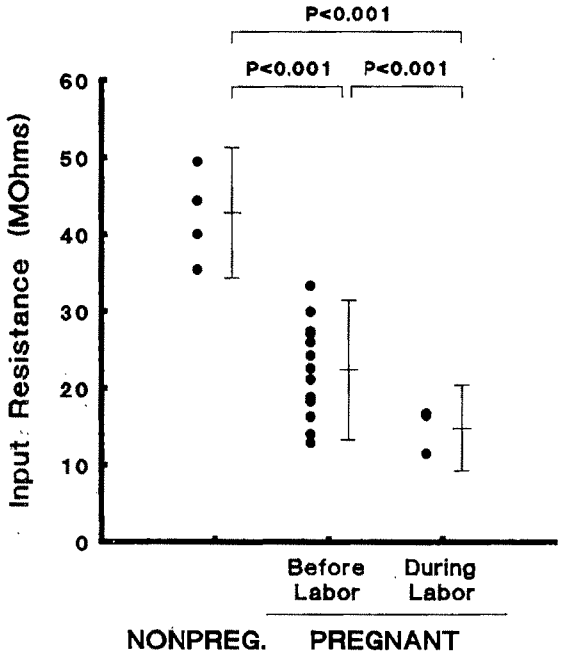


Fig. 2. Input resistances measured in smooth muscle cells of myometrial preparations from women. *Closed circles,* Average value from impaled cells of preparations obtained from same women; *bars,* mean values ± SEM obtained by combining all values from impaled cells of each group. *p* Values are analyzed by comparing values of input resistance from impaled cells between groups.

50 MΩ with 3 mol/L potassium chloride) with resistance of 150 to 200 MΩ. During impalement of the electrode into a single cell of a myometrial preparation, dye was injected with inward (hyperpolarizing) current pulses (5 to 50 nA, 200 msec/sec). The spread of Lucifer yellow from a source cell to neighboring cells was monitored under the microscope with epifluorescence optics (BP 450-490 excitation filter, RKP 510 dichronic mirror, and LP 515 barrier filter; Ernst Leitz). Lucifer yellow injection was terminated if values of membrane potential were markedly depolarized.

Drug treatment. All drug applications were conducted by exchanging the Tyrode's solution surrounding the tissue to the same solution mixed with one of the following agents: 1-octanol (1.2 mmol/L, Sigma) isoproterenol (10^{-6} mol/L, Sigma), or dibutyryl adenosine 3′,5′-cyclic monophosphate (5×10^{-4} mol/L, Sigma). Drug application was continued for 15 minutes, and then each solution was rapidly replaced twice with Tyrode's solution (washing).

Statistics. For the electrophysiologic measurements, results are mean ± SEM obtained by combining all values from impaled cells of preparations for each group. A one-way analysis of variance was used for comparisons of values among the nonpregnant group, the group before labor, and the group during labor, or among control, drug-treated, and washing groups. An unpaired Student *t* test was used to examine significant differences between groups. In some cases, the Mann-Whitney U test was used between groups with small numbers of samples. A p value <0.05 was considered statistically significant.

Results

Fig. 1 shows values of values of membrane potential of smooth muscle cells of human myometrial preparations obtained from a nonpregnant and two pregnant groups. There were no significant ($p = 0.303$ by analysis of variance differences in values of membrane potential among preparations from nonpregnant women (44.8 ± 6.52 mV [number of impaled cells = 25]), women before labor (44.4 ± 6.76 mV [105]), and women during labor (47.3 ± 6.86 mV [15]). Fig. 2 shows the input resistance values of myometrial cells in the above-mentioned three groups. There were significant ($p < 0.001$ by analysis of variance) differences in input resistance among these three groups. Input resistance was high in nonpregnant women (42.8 ± 8.52 mΩ [25]). However, input resistance was significantly ($p < 0.001$ by Student *t* test) reduced in women before labor (22.3 ± 9.06 MΩ [105]) and lowest in those during labor (14.8 ± 5.45 mΩ [15]). The input resistance in preparations from women during labor was significantly ($p < 0.001$ by Student *t* test) decreased compared with that before labor.

We applied a gap junction uncoupling agent, octanol, to further investigate the relationship between gap junctions and input resistance. Application of 1.2 mmol/L octanol caused an immediate relaxation in all myometrial preparations. Electric recordings showed that the values of membrane potential remained unchanged (nonpregnant p = 0.388, before labor p = 0.735, during labor p = 0.975 by analysis of variance) during octanol application and after washing in all groups (Fig. 3). However, input resistance was immediately (approximately 60 seconds after application of octanol) increased in pregnant groups (40.3 ± 7.90 MΩ before labor, 46.0 ± 3.04 MΩ during labor) to a similar high value to that in nonpregnant control preparations (Fig. 4). At 10 minutes after washing out the octanol, input resistance reversibly returned (19.8 ± 9.64 MΩ before labor, 14.4 ± 1.60 MΩ during labor) to the control value recorded before octanol application in the two pregnant groups. In contrast, the already higher input resistance in nonpregnant group was not significantly ($p = 0.962$ by analysis of variance) changed during octanol application (43.7 ± 4.40 MΩ) or after washing (42.3 ± 1.06 MΩ).

Injection of Lucifer yellow into a cell of myometrial preparations from women during labor (number of injections = 3) showed that the outline of the injected cells was illuminated brightly as an elongated spindle with the first two- to three-current pulses, and adjacent

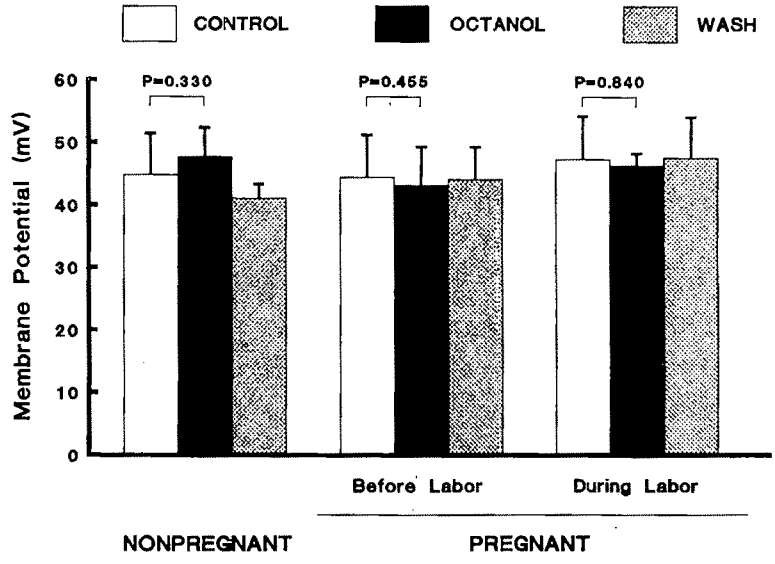


Fig. 3. Effect of octanol (1.2 mmol/L) on the membrane potential in myometrial preparations from nonpregnant and pregnant women. *Bars,* Mean values ± SEM obtained from impaled cells of preparations in various groups.

similarly shaped cells gradually became visible within a minute after the illumination of the source cell (Fig. 5, *A* and *B*). On the other hand, myometrial preparations from nonpregnant women[5] showed little or no spread of Lucifer yellow between the cells (Fig. 5, *C*). Only the injected source cell was illuminated, and Lucifer yellow was undetectable in adjacent cells even more than 10 minutes after injection, indicating that there was no Lucifer yellow transfer in nonpregnant myometrium. In the preparations from pregnant women before labor (14 cases), most injections (10 cases) showed good spread of Lucifer yellow similar to those during labor (Fig. 5, *D*), whereas some (4 cases) showed that although Lucifer yellow was detectable in adjacent cells, the spread was poorer (Fig. 5, *E*). In the pregnant preparations the result of Lucifer yellow injection during application of octanol was strikingly different from that before its application, with a marked decrease in transfer noted. As found in the nonpregnant controls, only the injected cell remained illuminated without spread to any other adjacent cells, indicating the Lucifer yellow transfer was blocked by octanol application (Fig. 5, *F*).

To determine whether elevated intracellular 3′,5′-cyclic monophosphate concentration had an influence on junctional communication between the myometrial cells, the preparations from nonpregnant women or pregnant women before labor were examined in the presence of 10^{-6} mol/L isoproterenol or 5×10^{-4} mol/L dibutyryl adenosine 3′,5′-cyclic monophosphate. In the pregnant group both of these two agents significantly (isoproterenol $p = 0.007$, dibutyryl adenosine 3′,5′-cyclic monophosphate $p < 0.001$ by Student t test) hyperpolarized values of membrane potential (isoproterenol 52.8 ± 4.65 mV, dibutyryl adenosine 3′,5′-cyclic monophosphate 54.6 ± 8.26 mV) from the control value (Fig. 6). These hyperpolarized values of membrane potential values rapidly returned to the control values after washing (isoproterenol 38.8 ± 3.46 mV, dibutyryl adenosine 3′,5′-cyclic monophosphate 44.3 ± 5.69 mV). In contrast, values of membrane potential in nonpregnant group was not significantly (isoproterenol $p = 0.937$, dibutyryl adenosine 3′,5′-cyclic monophosphate $p = 0.697$ by analysis of variance) changed during isoproterenol (45.9 ± 3.01 mV) or dibutyryl adenosine 3′,5′-cyclic monophosphate (43.2 ± 6.26 mV) application and after washing. Input resistance of myometrial cells of the pregnant group was significantly (isoproterenol $p < 0.001$, dibutyryl adenosine 3′,5′-cyclic monophosphate $p < 0.001$ by Student t test) increased by application of either of these two agents (isoproterenol 42.9 ± 7.17 MΩ, dibutyryl adenosine 3′,5′-cyclic monophosphate 39.7 ± 6.78 MΩ) (Fig. 7). These high input resistance values returned to control values after washing. However, the already high input resistance of the nonpregnant group was not significantly (isoproterenol $p = 0.931$, dibutyryl adenosine 3′,5′-cyclic monophosphate $p = 0.666$ by analysis of variance) changed either during isoproterenol (42.8 ± 5.21 MΩ) or dibutyryl adenosine 3′,5′-cyclic monophosphate (39.5 ± 2.12 MΩ) application and after washing. Therefore the effects of these two agents on input resistance (i.e., electric coupling), only found in pregnant but not in nonpregnant groups, are similar to those of octanol. Again, Lucifer yellow transfer between the pregnant myometrial cells was blocked by application of either of these agents (data not shown).

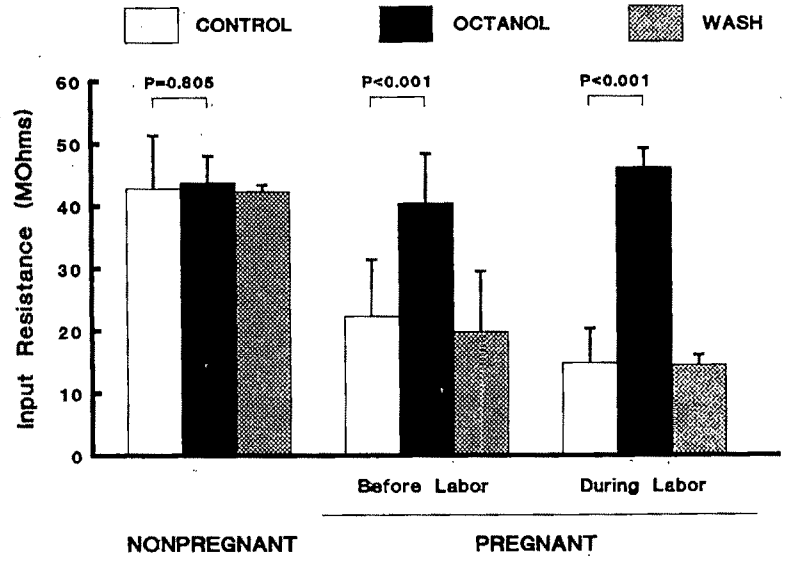


Fig. 4. Effect of octanol on the input resistance in myometrial preparations from nonpregnant and pregnant women. *Bars,* Mean values ± SEM obtained from impaled cells.

Comment

Direct measurement of the electric resistance (input resistance) between human myometrial cells shows a large decrease during pregnancy and a further decrease after the onset of labor. Intracellular injection of the fluorescent molecule Lucifer yellow demonstrates rapid and extensive spread from an injected cell to neighboring cells in the myometrial tissues from pregnant women but not in those from nonpregnant women. Moreover, octanol, isoproterenol, and dibutyryl adenosine 3′,5′-cyclic monophosphate indicate the rapid and reversible decrease in input resistance and Lucifer yellow transfer with changes requiring seconds to minutes. These results provide evidence that electric and metabolic coupling between human myometrial cells is dramatically increased during pregnancy, reaching a maximum at term during labor, and that this coupling is rapidly and reversibly decreased after specific forms of drug treatment. These results suggest two modes of modulation of gap junction coupling between smooth muscle cells of human myometrium during pregnancy, one regulating their presence and the other their permeability.

In rats and guinea pigs, morphologic studies have shown that gap junctions increased in number and size between myometrial cells at parturition, although somewhat higher numbers were present before parturition in the latter species.[1, 2, 4] On the other hand, recent studies of determination of input resistance and Lucifer yellow spread have demonstrated that electric and metabolic coupling was improved between myometrial cells in these species during periods of term labor.[10-12] In both species, because the improvement of the coupling was well correlated with development of gap junctions, it was concluded that the site of coupling and low electric resistance was the gap junctions and that the alteration in the coupling was based on the change in number of gap junctions between myometrial cells.

This study evaluated the change in gap junction number by showing the difference in electric and metabolic coupling between myometria from nonpregnant and pregnant women. The improved cell-to-cell coupling observed in pregnant groups provides evidence that gap junctions increase in myometrium from women undergoing cesarean section, although gap junction profiles in women during early pregnancy and normal, spontaneous vaginal delivery are not known. Furthermore, myometrial cells were found to be relatively well coupled in pregnant women even before labor, suggesting that the number of gap junction in pregnant human myometrium are already numerous before labor. This pattern of change in gap junction number is similar to that in guinea pigs and sheep[3, 4] but appear to be different from that in rats, where gap junctions increased dramatically about 24 to 36 hours before the onset of labor.[1, 2] The different patterns of change in structural coupling may be dependent on the changing hormonal environment in each species.

Because gap junctions are thought to provide a pathway for the flow of electrolytes and small molecules between cells,[13] the appearance of gap junctions would promote electrical and contractile synchrony in the myometrium, resulting in coordinated contractions of the uterus. Therefore their appearance between myometrial cells in human preceding or during labor is necessary for the generation of powerful contractions of the uterus and termination of pregnancy.

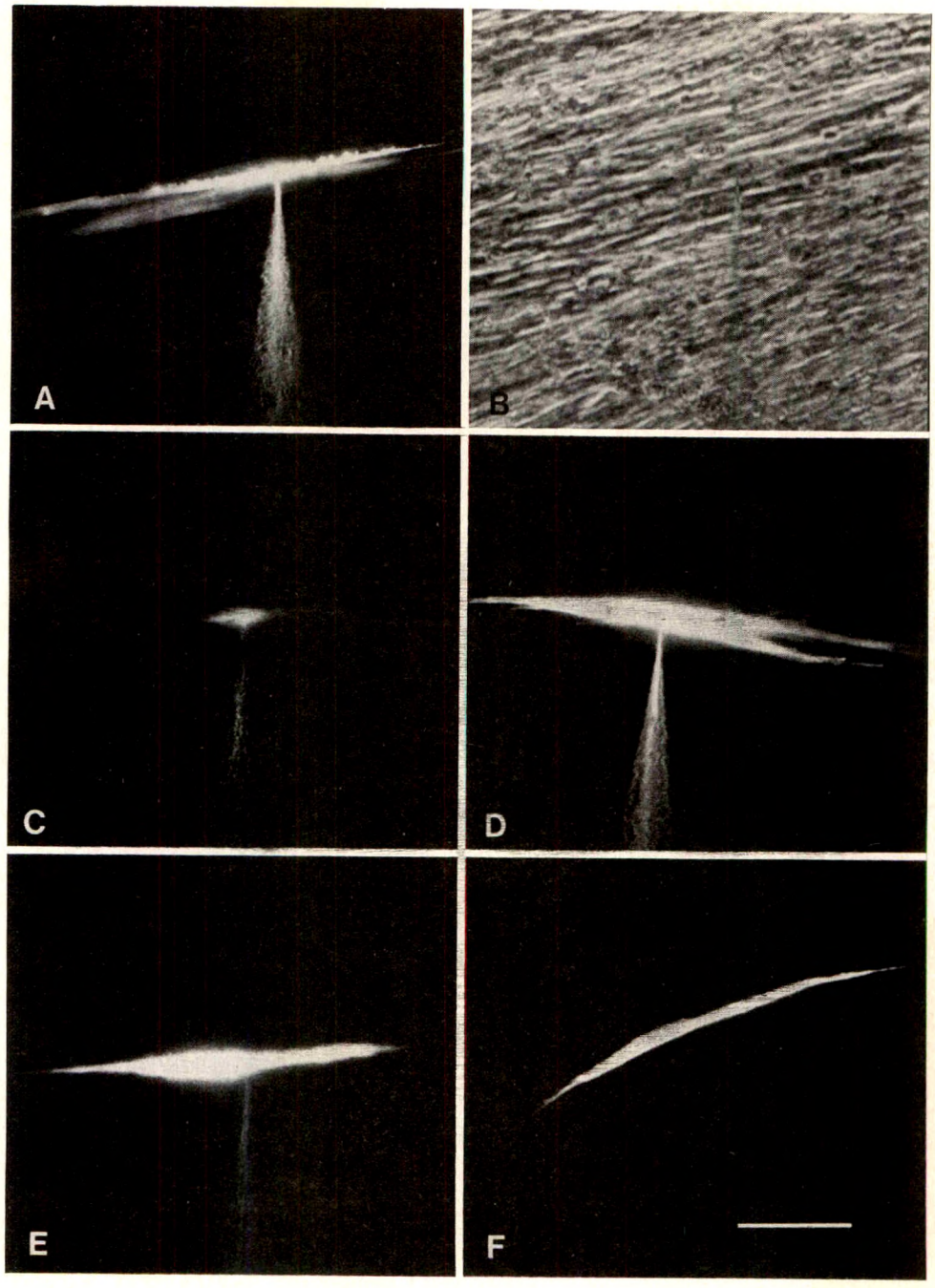


Fig. 5. Fluorescence photomicrographs after injecting the fluorescent probe Lucifer yellow into cells in myometrial preparations from women. *Scale bar* (in **F**) represents 100 μm. All six figures (**A** to **F**) are same magnification. **A,** Two minutes after injection of Lucifer yellow into cell in tissue from woman during labor. Lucifer yellow fluorescence is visible in several neighboring cells. **B,** Phase-contrast photomicrograph of field in **A. C,** Five minutes after injection of Lucifer yellow into cell in tissue from nonpregnant woman. Only injected cell is illuminated, and Lucifer yellow is undetectable in adjacent cells. **D,** Three minutes after injection of Lucifer yellow into cell in tissue from woman before labor. As found in tissue from woman during labor **(A),** Lucifer yellow spreads well to neighboring cells. **E,** Five minutes after injection of Lucifer yellow into cell in tissue from another woman before labor. Lucifer yellow is detectable in adjacent cells, but spread of Lucifer yellow is poorer than that found in **D. F,** Ten minutes after injection of Lucifer yellow into cell in 1.2 mmol/L octanol-applied tissue from woman during labor. Microelectrode was removed from cell at 5 minutes after injection. Lucifer yellow is detectable in only injected cell.

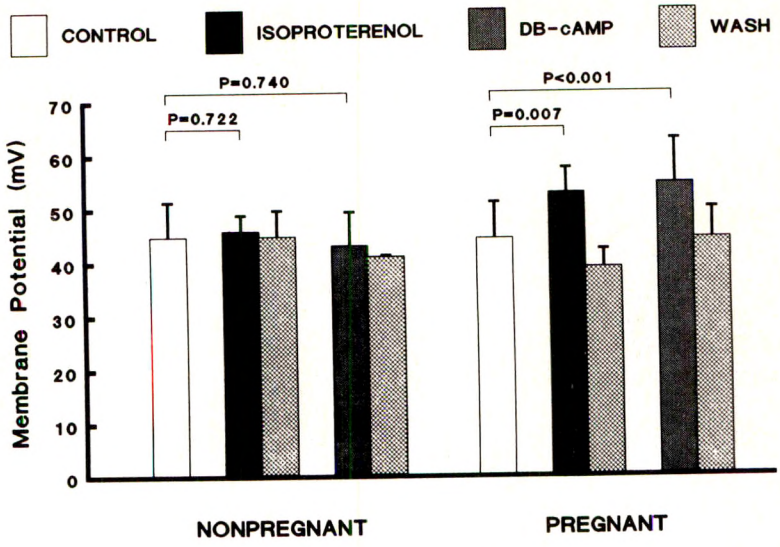


Fig. 6. Effect of isoproterenol (10^{-6} mol/L) or dibutyryl adenosine 3′,5′-cyclic monophosphate (5×10^{-4} mol/L) on the membrane potential in myometrial preparations from nonpregnant and pregnant women. *Bars*, Mean values ± SEM from impaled cells.

Not only is the presence of gap junctions necessary for cell-to-cell coupling, but openness of the channels is also required for electrolytes and metabolites to pass between myometrial cells. Experimentally, cell-to-cell coupling can be reversibly changed by the alteration of permeability of gap junctions, according to changes in intracellular Ca^{2+}, intracellular pH, intracellular 3′,5′-cyclic monophosphate, and many pharmacologic agents such as long-chain alcohols.[14-16] Opened (functionally well-coupled) gap junctions would be expected to promote greater electrical and contractile synchrony in the myometrium and lead to more forceful and frequent contractions and more effective labor. Alternatively, closed (functionally uncoupled) gap junctions would reduce the synchrony and lead to ineffective labor and a prolongation of pregnancy (i.e., the same condition existing in the absence of the junctions). The possible significant role of modulation of gap junction permeability in the myometrial contractility was observed in this study after the application of the agents (octanol, isoproterenol, and dibutyryl adenosine 3′,5′-cyclic monophosphate) that close gap junctions (Figs. 4 and 7).

Octanol is reported to decrease junctional permeability by decreasing the pore size of gap junctions, thereby resulting in uncoupling between cells.[16] This study has shown that octanol rapidly (≤60 seconds) increased input resistance and blocked dye spread after application to myometrial preparations from pregnant women, where gap junctions are numerous and input resistance was initially low before treatment. Furthermore, input resistance returned to pretreatment values immediately after washing out octanol from the preparations. These quick changes in input resistance at both application of and recovery from octanol indicate that this alcohol rapidly and reversibly regulates gap junction permeability, because such a rapidity cannot be explained by changes in gap junction numbers. On the other hand, octanol affected neither input resistance nor dye spread in preparations from nonpregnant women, indicating that octanol has little effect on tissues with no or few gap junctions between cells.

As found during application, octanol, isoproterenol, and dibutyryl adenosine 3′,5′-cyclic monophosphate in myometrial preparations from pregnant women increased input resistance and blocked dye spread with changes requiring similar time scales. The decreased coupling by these agents results from the decrease in junctional permeability, because their changes and predominant effects on the pregnant tissue are identical to those observed during application of octanol (Fig. 4 vs Fig. 7). Although the sites on which these two agents act are different, both of them are well known to elevate 3′,5′-cyclic monophosphate in the myometrial cells.[17, 18] This implies that, regardless of the sites, the uniform consequence of elevated 3′,5′-cyclic monophosphate is the reduction in permeability of gap junctions. Similar effects of elevated 3′,5′-cyclic monophosphate on junctional permeability has been shown in rat myometrium,[19] but the mechanisms by which 3′,5′-cyclic monophosphate reduces the permeability of myometrial gap junctions is unclear. Recently it has been proposed that activation of protein kinase by 3′,5′-cyclic monophosphate with consequent phosphorylation of junctional

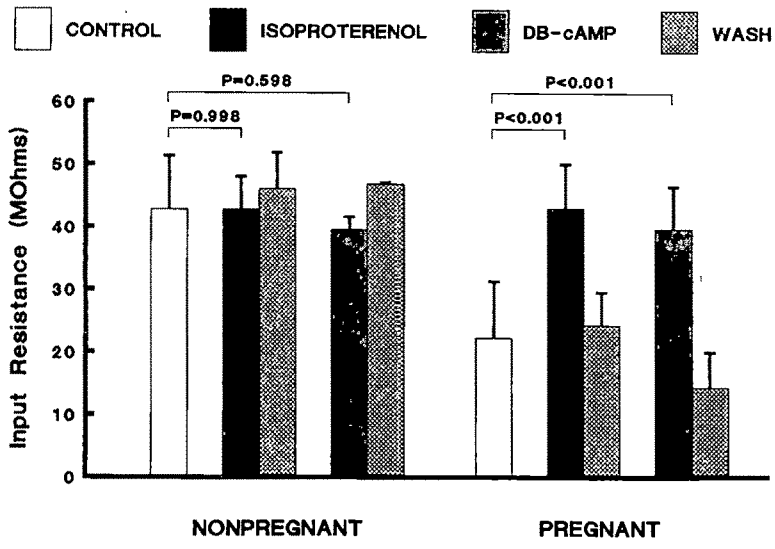


Fig. 7. Effect of isoproterenol or dibutyryl adenosine 3′,5′-cyclic monophosphate on input resistance in myometrial preparations from nonpregnant and pregnant women. *Bars*, Mean values ± SEM from impaled cells.

protein is necessary for the change in gap junction permeability in rat heart and liver,[20, 21] but the functional significance of this remains to be clearly identified.

Isoproterenol is one of the nonselective β-adrenergic receptor agonists. In clinical treatment other β-adrenergic receptor agonists with fewer side effects (primarily β_2-adrenoceptor agonists) are most widely used as tocolytic drugs.[22] They are reported to stimulate adenylate cyclase and thereby indirectly elevate 3′,5′-cyclic monophosphate in the myometrial cell.[23] Therefore 3′,5′-cyclic monophosphate is thought to play an important role in the modulation of uterine contractility. However, the exact mechanisms by which it inhibits spontaneous activity in the myometrium is poorly understood and remains controversial. It has been suggested to reduce intracellular Ca^{2+} concentration either by stimulating Ca^{2+} uptake into storage sites[24] or by increasing Ca^{2+} efflux across the plasma membrane.[25] However, the closure of gap junctions could produce more profound effects on relaxation than its direct action on Ca^{2+}. Closure of the junctions would prevent exitation by inhibiting the spread of action potentials and also thereby indirectly inhibit Ca^{2+} entry. One of the primary sites of action for agents that stimulate 3′,5′-cyclic monophosphate in myometrial cells may be the closure of gap junctions and the promotion of the ineffective spread of electrical activity between myometrial cells. In addition, drugs such as the β-adrenergic receptor agonists hyperpolarize the values of membrane potential (Fig. 6), thus further reducing excitability. However, hyperpolarization is clearly not a requirement for decreased excitability, because octanol produces uncoupling independent of any change in values of membrane potential (Fig. 3).

We found that spontaneous contractile activity in the pregnant myometrial preparations was completely prevented by application of either octanol, isoproterenol, or dibutyryl adenosine 3′,5′-cyclic monophosphate. The drug-induced quiescence in vitro may be indirect evidence that increased permeability of gap junctions is necessary for improvement of coupling and propagation of activity. These data are further interpreted to mean that the modulation of junctional permeability may be an important mechanism, both physiologically and pharmacologically, for regulation of myometrial contraction, especially in species where a relatively high number of gap junctions exist before labor. The present study demonstrates that input resistance in myometrial tissues from women before labor is already low and indicates that gap junctions exist even before labor. However, this low value of input resistance in the nonlabor group may be an artificially low in vitro. There might be some endogenous substance that keeps the junctions in the closed configuration until just before labor in vivo. This substance may not be present in vitro, and thus most gap junctions may be in open state.

The findings of these studies suggest that cell-to-cell coupling between human myometrial cells is regulated by both the presence and the permeability of gap junctions and that these two methods of modulation of GJs may be major mechanisms for control of myometrial contractile activity in the human uterus during pregnancy. Further studies are needed to clarify the role of gap junctions in human labor, especially normal vaginal

deliveries, and to assess clinical treatment with excitants and inhibitors of uterine contractions in relation to modulation of gap junctions.

We thank Ms. Debbie Merrett for technical assistance.

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Effects of danazol at the immunologic level in patients with adenomyosis, with special reference to autoantibodies: A multi-center cooperative study

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OBJECTIVE: We attempted to evaluate the effects of danazol on autoantibodies, in particular, to phospholipids, and on the immune system in patients with adenomyosis.

STUDY DESIGN: Forty-two patients with adenomyosis who had high titers of autoantibodies were randomly chosen, and they received 400 mg/day of danazol for 4 months (n = 22) or underwent hysterectomy (n = 20).

RESULTS: Among the six autoantibodies we investigated, the incidence of antiphosphatidylinositol immunoglobulin G was the highest (42.9%), followed by antiphosphatidylglycerol immunoglobulin G (38.1%). The autoantibody titers decreased with time and were comparable to the control values 16 weeks after treatment in both groups. Total serum levels of immunoglobulin G and M were high before treatment, but immunoglobulin M levels decreased significantly in week 8 during treatment with danazol, whereas C4 levels increased and C3 levels decreased with danazol.

CONCLUSION: Danazol has an inhibitory effect on the autoimmunologic response associated with adenomyosis. (Am J Obstet Gynecol 1992;167:481-6.)

Key words: Adenomyosis, endometriosis, autoantibody, autoimmunity, danazol

Endometriosis is associated with immunologic abnormalities such as increases in the number[1] and activity[2] of macrophages in peritoneal fluid and secretion of cytokines such as interleukin-1 and tumor necrosis factor by these cells.[3, 4] In addition, autoantibodies, in particular, antiphospholipid antibodies, are often detected in peripheral blood samples obtained from patients with endometriosis.[5-7] Antiphospholipid antibodies, which are found in many patients with endometriosis, are often detected in patients with autoimmune diseases such as systemic lupus erythematosus[8] and rheumatoid arthritis.[9] More recently, autoantibodies have also been demonstrated in women with repeated spontaneous abortion.[10, 11]

Danazol, a drug commonly used for the treatment of endometriosis, exerts a direct effect on endometriosis,[12] reduces autoantibodies, and normalizes the immunologic abnormalities associated with it.[13] In a previous study we reported that autoantibodies, in particular, antiphospholipid antibodies, are also often detected in peripheral blood samples obtained from patients with adenomyosis.[6, 7] In the current study we administered danazol to patients with adenomyosis who were positive for autoantibodies to evaluate its effects on autoantibodies and the immune system.

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Material and methods

Subjects. Fifty-nine patients with adenomyosis diagnosed by bimanual examination, ultrasonography, hysteroscopy,[14] serum CA 125 levels, or autoantibody levels were included in this study. Bimanual examination and ultrasonography were performed to ascertain the size of the uterus and to detect adenomyoma. In a previous study a retrospective analysis revealed that 93.8% of the patients who underwent hysterectomy and in whom the presence of adenomyosis was histologically confirmed had elevated levels of autoantibodies and/or CA 125 in peripheral blood.[7] Further, hysteroscopy revealed that 31 (77.5%) of the 40 patients with adenomyosis had characteristic findings such as a persistent large glandular ostium and/or network of dilated vessels throughout the menstrual cycle.[14]

Autoantibody levels were increased in 42 cases that were divided into two groups in a randomized fashion: 22 patients received danazol (400 mg/day) for 16 weeks and 20 underwent total hysterectomy (Table I). There

Table I. Summary of clinical parameters in surgery and danazol groups

	Surgery group	*Danazol group*
Clinical condition of patients with high autoantibody titers	20	22
Adenoymosis	9/20	11/22
Adenoymosis + adenomyoma	7/20	6/22
Adenoymosis + endometriosis	3/20	3/22
Adenoymosis + adenomyoma + endometriosis	1/20	2/22
Diagnosis		
Elevated CA 125 (≥50 U/ml)	11/20	13/22
Ultrasonography	13/20	16/22
Hysteroscopy	8/11	8/12
Symptoms		
Dysmenorrhea	18/20	21/22
Hypermenorrhea	18/20	21/22
Pelvic pain	19/20	19/22
Low back pain	11/20	12/22
Signs		
Enlarged uterus (≥165 gm)	19/20	17/22

Table II. Changes in lymphocyte subsets

Group	*$CD4^+4B4^+$* (%)*	*$CD4^+4B4-$† (%)*	*$CD11-CD8^+$‡ (%)*	*$CD11^+CD8-$§ (%)*	*$CD11^+CD8^+$‖ (%)*	*$CD4^+4B4^+/CD11^+CD8^+$¶*
Surgery group						
Before treatment ($n = 10$)	27.2 ± 12.0	14.8 ± 6.2	17.9 ± 4.3	19.2 ± 7.2	4.9 ± 3.7	7.5 ± 3.9
8 wk ($n = 7$)	26.5 ± 5.1	19.6 ± 6.2	18.2 ± 4.2	18.3 ± 5.8	5.8 ± 2.6	5.7 ± 3.1
16 wk ($n = 5$)	23.5 ± 7.4	15.8 ± 2.5	19.0 ± 4.6	16.8 ± 3.5	6.7 ± 2.4	4.1 ± 1.7
Danazol group						
Before treatment ($n = 11$)	26.0 ± 7.4	19.4 ± 5.2	20.0 ± 5.7	18.0 ± 3.5	5.1 ± 2.1	6.3 ± 3.6
8 wk ($n = 10$)	25.3 ± 3.8	17.4 ± 6.1	20.1 ± 5.2	18.1 ± 5.5	5.4 ± 1.8	5.4 ± 2.3
16 wk ($n = 8$)	21.9 ± 4.4	18.8 ± 6.0	22.7 ± 6.0	16.4 ± 4.6	5.8 ± 1.3	4.1 ± 1.1
Control group ($n = 45$)	22.6 ± 5.9	17.8 ± 5.8	24.3 ± 6.1	15.9 ± 6.5	5.8 ± 2.8	4.9 ± 3.1

*$\chi^2 = 6.09$.
†$\chi^2 = 3.86$.
‡$\chi^2 = 12.46$, $p < 0.1$.
§$\chi^2 = 6.48$.
‖$\chi^2 = -48.54$.
¶$\chi^2 = 6.51$.

were 19 patients with an enlarged uterus (>165 gm) in the surgery group and 17 in the danazol group (calculated from ultrasonography). Postoperative histologic examinations confirmed the presence of ectopic endometrial tissues in the myometrium in all cases from the surgery group. The histologic diagnosis of adenomyosis was based chiefly on the finding of endometrial islands deep beneath the mucous surface, within the myometrium; that is, when the endometrial tissue was at least the distance of one high-power field below the basal endometrium. The diagnosis of endometriosis was established by laparoscopy or laparotomy. Sixty-six normal women who had ligation of fallopian tubes for contraception purposes at laparoscopy and who were without adenomyoma or inflammation of pelvic tissues served as controls. Women without adenomyosis and patients with adenomyosis and a history of repeated spontaneous abortion, thrombosis, or autoimmune diseases or who were receiving medication were excluded.

Assay methods. Blood samples were collected before treatment and 8 and 16 weeks after operation in one group and during treatment in the danazol group for immunologic evaluation. Antibodies to the following phospholipids were determined by enzyme-linked immunosorbent assay[6,7]: anticardiolipin immunoglobulin (Ig) G and IgM (CL-G and CL-M), anti–phosphatidic acid IgG (PA-G), antiphosphatidylinositol IgG (PI-G), antiphosphatidylserine IgG (PS-G), and antiphosphatidylglycerol IgG (PG-G). Briefly, 20 μg/ml of each phospholipid (Sigma Chemical Co., St. Louis) was allowed to adsorb after immobilizing protamine sulfate (5 μg/ml, Sigma) on microplates. Fiftyfold diluted sera were then added and allowed to react with peroxidase-labeled antihuman IgG and IgM antibodies (Dako, Denmark). After coloration with o-phenylenediamine

dihydrochloride (Sigma), reaction was stopped by adding 100 μl of 2.5 mol/L sulfuric acid and absorbance was determined at 490 nm. Standard sera supplied by Harris[15] were used for quantitative determination. IgG and IgM levels were expressed in GPL and MPL units per milliliter, respectively. Values exceeding mean + 2 SD of normal were regarded as abnormally high.

Immunoglobulins (IgG, IgM, and IgA) and components C3 and C4 of complement in serum were determined by latex agglutination photometric assay.[16] The fully automatic immunochemical analyzer used to process the samples (laser nephelometry, LX-3000; Eiken Chemical Co., Ltd., Tokyo) can perform all procedures from imputing of parameters to data printout completely automatically. However, dilution of the samples before the analysis was done manually with a special diluter. Absorbance was determined at 660 nm, 6 and 33 seconds after the diluted test sera were mixed with antiserum (Eiken) in cells for reaction, and the difference between the two measurements was calculated. Concentrations were calculated from the standard curve.

Lymphocyte subsets were determined by two-color flow cytometry.[17] Monoclonal antibodies CD4-FITC (Becton Dickinson Immunocytometry Systems, San Jose, Calif.), 4B4-RD1 (Coulter Electronics, Hialeah, Fla.), CD11-FITC (Coulter), and CD8-PE (Becton Dickinson) were used to determine lymphocyte subsets with a laser flow cytometer (FCM-1D; Nihon Bunko, Tokyo): $CD4^+4B4^+$ (helper T cells), $CD4^+4B4^-$ (suppressor/inducer T cells), $CD11^-CD8^+$ (cytotoxic T cells), $CD11^+CD8^-$ (natural killer T cells), and $CD11^+CD8^+$ (suppressor T cells).

Serum CA125 levels were determined with radioimmunoassay kits (Centocor, Tokyo). Radioimmunoassay kits for serum estradiol and free testosterone levels were purchased from DPC Japan (Tokyo).

Statistical analyses. The results were expressed as mean ± SD. Statistical analyses of the immunoglobulins, complement components, and serum steroid hormone levels were performed with the one-way analysis of variance, and the levels of lymphocyte subsets were determined with the Kruskal-Wallis test. Differences were regarded as significant if $p < 0.05$.

Results

The incidences of each autoantibody in the control ($n = 66$) and adenomyosis ($n = 42$) groups are shown in Fig. 1. The incidence of autoantibody in the control group ranged between 2.5% and 6.7%; among them, that for PA-G was the highest. In the adenomyosis group the incidence was highest for PI-G (18 patients, 42.9%), followed by PG-G (38.1%) and PS-G (31.1%), and then by CL-M, PA-G, and CL-G. The number of types of autoantibodies per patient was 1 for the majority of patients (21 patients, 50.0%), followed by 2 in 10 patients (23.8%) and 3 in 6 patients (14.3%).

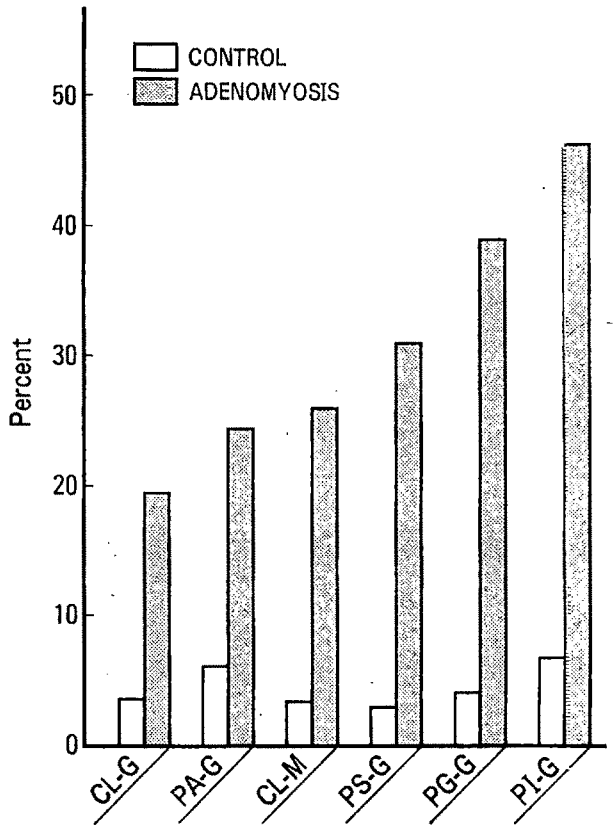


Fig. 1. Incidence of different types of autoantibodies in control and adenomyosis groups.

Fig. 2 illustrates the changes in individual autoantibodies during medical treatment and after surgery. The levels of autoantibodies in the control group were as follows: 0.97 ± 0.37 GPL U/ml for CL-G, 1.92 ± 1.89 MPL U/ml for CL-M, 1.37 ± 0.09 GPL U/ml for PA-G, 1.09 ± 0.39 GPL U/ml for PI-G, 0.70 ± 0.51 GPL U/ml for PS-G, and 1.25 ± 0.13 GPL U/ml for PG-G. The overall mean ± SD of autoantibodies in all patients with adenomyosis (surgery and danazol groups combined) before treatment was as follows: 6.27 ± 3.96 GPL U/ml for CL-G, 12.48 ± 5.07 MPL U/ml for CL-M, 8.42 ± 7.37 GPL U/ml for PA-G, 5.85 ± 2.55 GPL U/ml for PI-G, 9.71 ± 8.56 GPL U/ml for PS-G, and 4.48 ± 0.67 GPL U/ml for PG-G. All values had decreased at week 8 of treatment in both groups and were very close to control values by week 16. At week 8 the number of patients with high titers of autoantibodies was 12 in each group, that is, 60.0% in the surgery group and 54.5% in the danazol group (Fig. 3). At week 16 it had decreased to 6 (30.0%) in the surgery group and only 3 (13.6%) in the danazol group. The number of patients with high titers of autoantibodies thus markedly decreased with time.

Table II shows the changes in lymphocytes subsets observed after treatment. Levels of helper T cells, which were higher than the upper control limit before

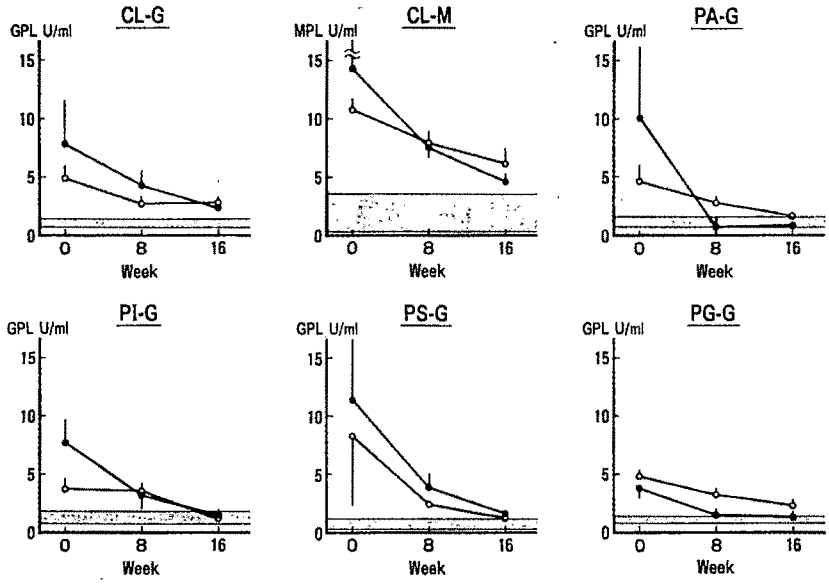


Fig. 2. Changes in autoantibodies (mean ± 2 SD) in surgery (○) and danazol (●) groups. *Shadowed area* indicates control range.

Table III. Changes in immunoglobulin and complement component levels

Group	*IgG (mg/dl)*	*IgM (mg/dl)*	*IgA (mg/dl)*	*C3 (mg/dl)*	*C4 (mg/dl)*
Surgery group					
Before treatment (n = 9)	1543 ± 270*	225 ± 85÷	255 ± 91	92 ± 11	25 ± 4
8 wk (n = 7)	1633 ± 159	283 ± 129‡§	236 ± 74	76 ± 17	23 ± 3
16 wk (n = 6)	1613 ± 173	283 ± 164	245 ± 81	89 ± 9*	26 ± 3
Danazol group					
Before treatment (n = 16)	1322 ± 244‖	224 ± 61†	253 ± 79	76 ± 12	26 ± 8
8 wk (n = 14)	1276 ± 309	191 ± 62*‡	218 ± 70	66 ± 6†¶	31 ± 6#
16 wk (n = 11)	1312 ± 262	184 ± 75	216 ± 65	69 ± 5	31 ± 8#
Control group (n = 66)	1177 ± 224	154 ± 58	208 ± 82	80 ± 13	25 ± 7

*$p < 0.05$ (vs control).
†$p < 0.001$ (vs control).
‡$p < 0.05$ (vs pretreatment value).
§$p < 0.001$ (vs pretreatment value).
‖$p < 0.01$ (vs control).
¶$p < 0.01$ (vs pretreatment value).
#$p < 0.02$ (vs control).

treatment in both groups, tended to decrease; however, the difference at week 16 was not significant. In contrast, suppressor T cells, which showed slightly low values compared with control before treatment, tended to increase with time. The helper/suppressor T-cell ratio decreased linearly to values that were slightly lower than those of the control population. Cytotoxic, suppressor/inducer, and natural killer T cells remained almost unchanged in both groups.

Table III shows changes in values of immunoglobulin and complement components C3 and C4 after treatment. Pretreatment IgG levels were significantly higher in the surgery and danazol groups than in the control group ($p < 0.05$ and $p < 0.01$). However, no apparent change was seen after treatment in either group. Pretreatment IgM values were also significantly higher in both groups than in the control ($p < 0.001$). In the danazol group at week 8 these values had significantly decreased as compared with pretreatment values ($p < 0.05$) and were almost at control levels at week 16. IgA values were close to the control upper limit before treatment in both groups. Slight decreases were seen after treatment in both groups.

C3 levels were comparable to those in the controls in both groups before treatment. In the danazol group they had significantly decreased compared with the pretreatment values at week 8 ($p < 0.01$). C4 levels were also comparable to those in the controls in both

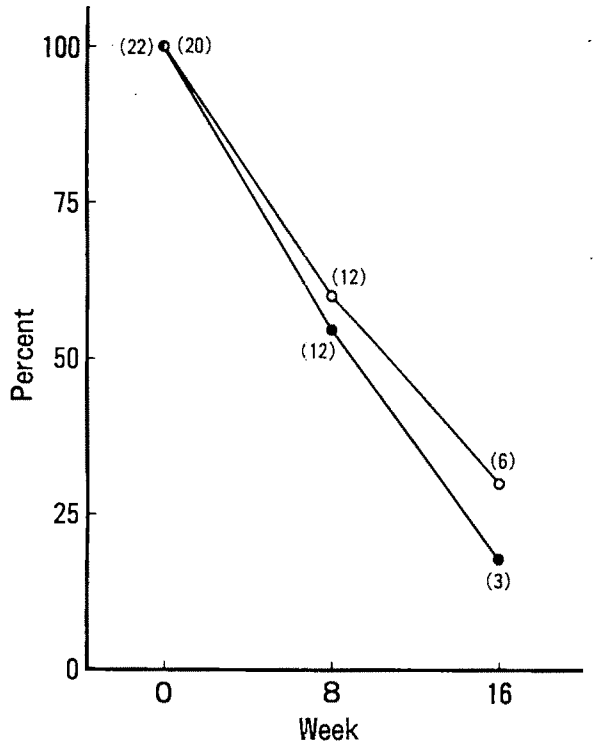


Fig. 3. Percentage of patients with high titers of autoantibody in surgery (○) and danazol (●) groups. *Figures in parentheses* indicate number of patients.

groups before treatment. However, in contrast to C3 levels, C4 significantly increased compared with the control values at weeks 8 and 16 in the danazol group ($p < 0.001$).

Serum estradiol and free testosterone levels were 32 ± 16 and 1.1 ± 0.9 pg/ml, respectively, in the control group (Table IV). Estradiol levels tended to decrease during treatment with danazol, but the difference was not significant. In contrast, free testosterone levels markedly increased during treatment, showing 31- and 34-fold increases over pretreatment levels at weeks 8 and 16, respectively ($p < 0.001$).

Comment

Endometriosis is associated with immunologic abnormalities, and high titers of autoantibodies have often been detected in serum obtained from patients with this condition,[5-7] suggesting that an autoimmune process is involved in endometriosis. El-Roeiy et al.[18] administered danazol to patients with endometriosis and high autoantibody titers and observed a decrease of autoantibodies and immunologic improvement with time during treatment. In a previous study we reported that high titers of autoantibodies were detected in patients with adenomyosis as often as in those with endometriosis.[6, 7] In this study in patients with adenomyosis we show that autoantibody levels decreased with time during treatment and reached almost control values after 16 weeks of treatment. This effect of danazol

Table IV. Changes in serum estradiol and free testosterone levels

Group	*Estradiol (pg/ml)*	*Testosterone (pg/ml)*
Control group (n = 14)	32 ± 16	1.1 ± 0.9
Danazol group		
Before treatment (n = 17)	33 ± 17	1.0 ± 1.0
8 wk (n = 12)	23 ± 14	30.5 ± 11.1*
16 wk (n = 10)	26 ± 7	33.8 ± 12.0*

*$p < 0.001$ (vs pretreatment value).

is similar to that reported in patients with endometriosis.[18]

Findings obtained in the current study show that danazol has an effect on immunoglobulin concentrations, which were higher before treatment in patients with adenomyosis than in the controls. According to El-Roeiy et al.,[18] IgG and IgM levels are higher in autoantibody-positive patients with endometriosis than in those who are autoantibody negative. In the current study IgG and IgM levels tended to increase in the surgery group: in particular, increases in IgM levels were statistically significant compared with pretreatment values, 8 weeks after surgery. In contrast, immunoglobulin levels decreased after treatment in the danazol group. Especially, decreases in IgM levels were statistically significant compared with pretreatment values at week 8 during treatment. These findings corroborate in part those of El-Roeiy et al.[18] and indicate that danazol inhibits the production of immunoglobulins.

Only a few studies have been conducted on the effect of danazol on complement components in endometriosis. According to Agnello et al.,[19] C4 levels had increased in a patient with systemic lupus erythematosus during treatment with danazol. In the current study serum C3 and C4 levels responded differently to danazol therapy, with C3 levels decreasing significantly at week 8 during treatment compared with pretreatment levels and C4 levels increasing during treatment. Increases in C4 levels were not significant compared with pretreatment levels but were significant compared with controls. There are reports that C3 levels decrease in patients with autoimmune hemolytic anemia[20] and increase in those with hereditary angioedema[21] during treatment with danazol. Danazol has already been shown to affect immunocytes.[22, 23] C3 and C4 are produced by hepatocytes and immunocompetent cells such as monocytes and macrophages.[24] The fact that these complement components were affected differently by danazol in the current study suggests that danazol may act differently on those cells.

The fact that immunocompetent cells are under the influence of various hormones, in particular, steroid hormones, has been reported.[25] In fact, steroid

hormones such as estrogen,[26] androgen,[27] and glucocorticoids[28] have direct effects on T cells in vitro. In addition, estrogen increases helper T-cell activity and decreases suppressor T-cell activity[29] while androgen increases suppressor T-cell activity. These sex steroids also have direct effects on B cells.[30] In the current study estradiol levels decreased slightly on administration of danazol, while free testosterone levels markedly increased. However, the helper/suppressor T-cell ratio tended to normalize. Therefore it is uncertain if the changes in sex steroids may have affected the lymphocyte subsets.

It would be very interesting to know the mechanisms by which danazol affects the autoimmune response in patients with adenomyosis. The putative mechanisms of action of danazol include a direct effect on endometriosis tissues in addition to inhibitory effects on the hypothalamus, pituitary, and ovaries,[12, 31] resulting in regression of the endometriotic lesions. IgG and complement components C3 and C4 are very often deposited in ectopic endometriosis tissues in the tunica muscularis in patients with adenomyosis,[32] a finding that suggests that strong local immunologic reactions take place in these patients. It is therefore tempting to say that danazol might also have a direct inhibitory effect on the local immune response in patients with endometriosis and adenomyosis. Further studies on the mechanisms by which danazol affects the immune response are needed to provide an answer to these questions.

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Serial measurements of platelet angiotensin II binding in two patients with hypertension in pregnancy: Case reports

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Serial estimations of platelet angiotensin II binding were made in two patients with hypertension developing in the third trimester of pregnancy. The fall in platelet angiotensin II binding that is seen in normotensive pregnancy did not occur in these two patients. (AM J OBSTET GYNECOL 1992;167:487-8.)

Key words: Angiotensin II, platelet membrane glycoproteins, pregnancy toxemia, renin-angiotensin system

Two patients were recruited to a longitudinal study ($n = 30$) of platelet angiotensin II binding in normotensive pregnancy. In one of these patients platelet angiotensin II binding was measured before conception. Hypertension developed subsequently, and thus they were excluded from the original study. Neither woman was known to have renal, metabolic, or cardiovascular disease. Both patients were receiving unrestricted sodium intake and on recruitment to the study had normal serum urea, creatinine, and electrolyte values. In both patients serial platelet counts and plasma creatinine and urate levels were measured, with serial estimations of platelet angiotensin II binding and plasma angiotensin II concentration being performed, as previously described.[1]

Case reports

Case 1. A 28-year-old normotensive nulliparous woman was initially studied on day 6 of the menstrual cycle. She conceived 3 months later and was then studied at intervals during her pregnancy. She remained normotensive (diastolic blood pressure <80 mm Hg, Korotkoff phase 4) until 32 weeks' gestation, when a blood pressure recording of 140/95 (Korotkoff phase 4) mm Hg was noted. The blood pressure subsequently stabilized; however, at 37 weeks' gestation it was recorded as 145/100 (Korotkoff phase 4) mm Hg and proteinuria (2+) was noted on dipstick testing for the first time. After hospital admission, the blood pressure remained elevated and significant proteinuria was confirmed (0.63 gm per 24-hour specimen). Labor was induced. Eight hours after artificial rupture of membranes and commencement of oxytocin infusion, the cervix remained only 2 cm dilated. A cesarean section was performed and a male infant weighing 2.64 kg was delivered. She recovered well postoperatively, and at 12 weeks post partum she was normotensive, with no proteinuria on dipstick testing (<0.1 gm per 24-hour specimen).

Case 2. A 26-year-old primiparous woman was initially studied at 11 weeks' gestation, at which time the blood pressure was 110/65 (Korotkoff phase 4) mm Hg. She remained normotensive (diastolic blood pressure <80 [Korotkoff phase 4] mm Hg) until 38 weeks' gestation, when the blood pressure was recorded as 140/100 (Korotkoff phase 4) mm Hg. No proteinuria was present. At 36 hours after hospital admission, the blood pressure remained elevated, and she went into spontaneous labor. A male infant weighing 3.02 kg was delivered vaginally. At 12 weeks post partum the blood pressure was 120/60 (Korotkoff phase 4) mm Hg.

Fig. 1 illustrates the serial values of platelet angiotensin II binding in both patients, for comparison with the median values that were found in a longitudinal study of normotensive pregnancy.[1] When the serial estimations of platelet count, plasma urate and creatinine concentrations, and plasma angiotensin II concentration were considered, no value of either patient was outside the interquartile range established for normotensive pregnancy.[1]

Comment

In normotensive pregnancy there is a diminution in platelet angiotensin II binding, with a significant reduction being apparent by the first trimester, which parallels the pregnancy-induced decrease in pressor responsiveness to infused angiotensin II.[1] In women with established hypertension in pregnancy, the increased levels of platelet angiotensin II binding, in comparison with levels in normotensive primigravid women, also parallels the increased angiotensin II pressor responsiveness found in hypertension in pregnancy.[2]

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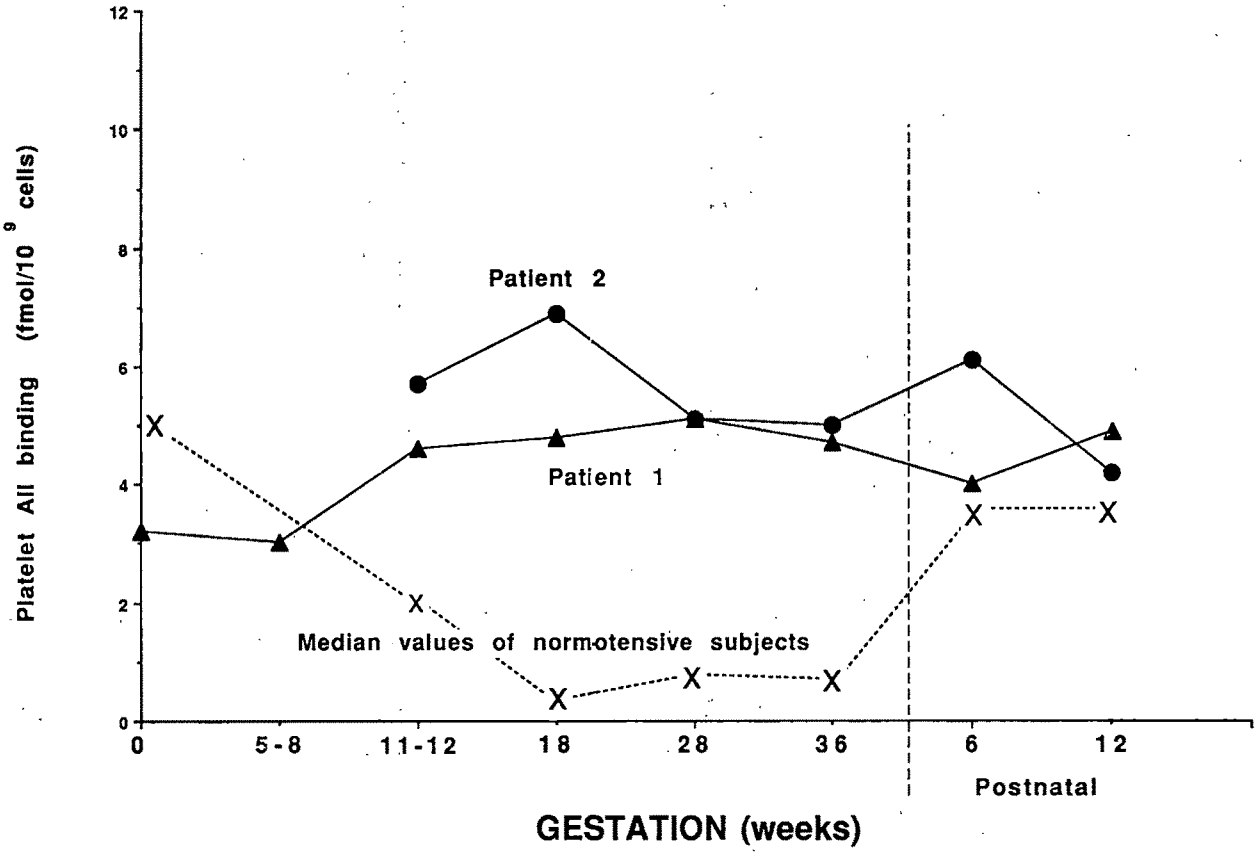


Fig. 1. Platelet angiotensin II binding in two patients in whom hypertension developed in pregnancy. Median values of platelet angiotensin II binding determined from a longitudinal study of normotensive pregnancy[1] are also shown.

The results from the two cases described support the hypothesis that the elevated levels of platelet angiotensin II binding in women with hypertension in pregnancy, as compared with those in normotensive primigravid women, are not due to an increase in binding toward the end of pregnancy in hypertensive patients but instead are due to absence of the early pregnancy decrease in platelet angiotensin II binding found in normotensive pregnancy. The use of platelet angiotensin II binding as a less invasive alternative to the angiotensin II sensitivity test in screening for hypertension in pregnancy has previously been suggested.[2] If the hypothesis above is correct, it should be possible to identify patients at risk of hypertension as early as the antenatal clinic booking visit.

Down-regulation by plasma angiotensin II concentration has been suggested as a mechanism controlling platelet angiotensin II binding in normotensive pregnancy.[1] The results of these two cases, in which platelet angiotensin II binding appeared to be unrelated to plasma angiotensin II concentration, suggest that a more complex mechanism regulates platelet angiotensin II binding in pregnancies complicated by hypertension.

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Ifosfamide alone and in combination in the treatment of refractory malignant gestational trophoblastic disease

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OBJECTIVE: We attempted to evaluate the use of ifosfamide either alone or in combination in patients with refractory malignant gestational trophoblastic disease.

STUDY DESIGN: Our study comprised, in part, a phase II multiinstitutional trial of ifosfamide in refractory gynecologic malignancies and, in part, a review of institutional experience with ifosfamide in combination chemotherapy.

RESULTS: Single-agent ifosfamide produced a significant response in titer in one of two patients with refractory choriocarcinoma. Ifosfamide with etoposide and cisplatin (also known as VIP) resulted in significant response in human chorionic gonadotropin titers in three patients with highly refractory metastatic gestational trophoblastic disease and one cure in this group of patients.

CONCLUSION: Ifosfamide has activity in refractory choriocarcinoma and, when combined with etoposide and cisplatin (VIP), may be curative. (Am J Obstet Gynecol 1992;167:489-95.)

Key words: Ifosfamide, refractory gestational trophoblastic disease

Metastatic gestational trophoblastic disease that carries a poor prognosis is cured in approximately 80% of patients receiving chemotherapy with drug combinations such as MAC (methotrexate, dactinomycin, and chlorambucil)[1] or EMACO (etoposide, methotrexate, dactinomycin, cyclophosphamide, and vincristine).[2] However, the development of drug resistance still accounts for a large proportion of the deaths from this illness.[3]

Ifosfamide, a congener of cyclophosphamide, has a broad spectrum of activity against tumors such as ovarian and cervical carcinomas and uterine sarcomas. Prohibitive urothelial toxicity requires the concurrent administration of mercaptoethane sodium sulfonate (mesna). In 1989, ifosfamide and mesna received Food and Drug Administration approval for use in refractory testicular carcinoma. This approval was based mainly on the work of Loehrer et al.[4] who reported sustained remissions in 7 of 48 men with such malignancies after treatment with etoposide, ifosfamide, and cisplatin (also known as VIP).

In a Gynecologic Oncology Group phase II study of ifosfamide, highly resistant malignant trophoblastic disease was treated in two patients. A favorable response in one of these patients led to the use of VIP in three additional women with refractory trophoblastic disease. These four cases are summarized here and in Figs. 1 through 4.

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Case reports

Case 1. A 27-year-old black woman underwent craniotomy for evaluation of a mass in March 1986, 5 months after the delivery of a viable preterm infant. Metastatic gestational choriocarcinoma was identified at the time of surgery. Staging studies indicated the presence of pulmonary metastases and an elevated serum human chorionic gonadotropin (hCG) level. She received chemotherapy with MAC over the next 8 months until the level failed to respond. She underwent two thoracotomies in December 1986 and a hysterectomy in January 1987. From this time until January 1988 she received chemotherapy with cisplatin and etoposide and then with methotrexate, hydroxyurea, vincristine, cyclophosphamide, and 5-fluorouracil. The hCG level rose to 5079 mIU/ml, and computerized tomography disclosed multiple new hepatic metastases. She received ifosfamide, 1.2 gm/m^2 intravenously daily for 5 days with mesna, 240 mg/m^2 intravenously im-

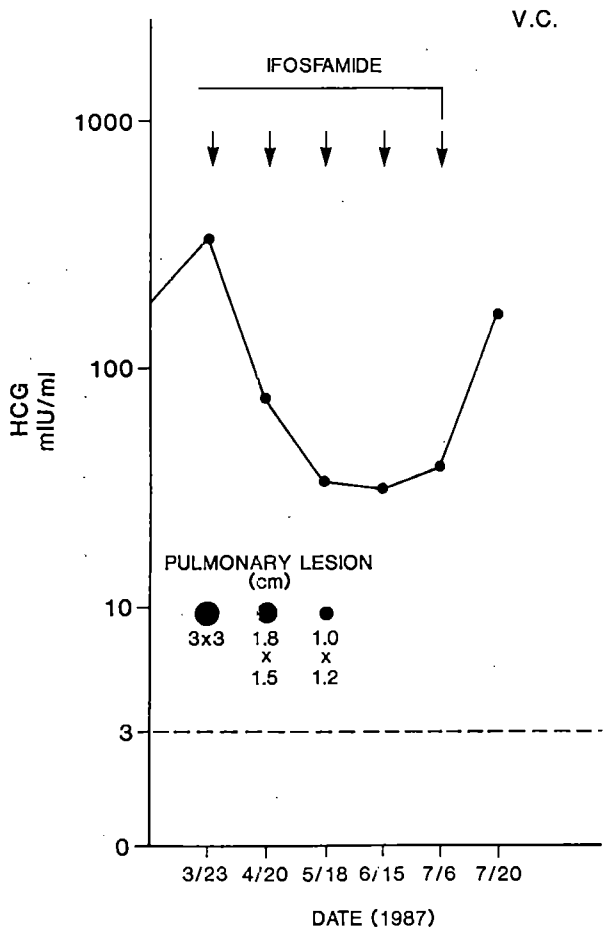


Fig. 1. hCG level and lesion response to ifosfamide alone in case 2.

mediately and 4 and 8 hours after each ifosfamide dose. The white blood cell count fell to 2300/mm³ and there was no other toxicity, but the hCG level rose to 49,690 mIU/ml within 4 weeks. This patient died 2 months later of respiratory failure.

Case 2. In a 30-year-old white woman, hemoptysis and vaginal bleeding developed about 4 months after the delivery of her second child by cesarean section. A curettage was done in July 1986 and choriocarcinoma was identified. Diffuse nodularity was observed on a chest radiograph. She received a single course of MAC, and the hCG level rose from 4786 to 7400 mIU/ml. She then was treated with eight courses of dactinomycin, etoposide, and methotrexate. This resulted in a fall in the hCG level to a nadir of 83 mIU/ml before it rose to 126 mIU/ml. She received two cycles of vinblastine, bleomycin, and cisplatin in November and December of 1986 and had a transient response in hCG. She underwent left thoracotomy and resection of pulmonary nodules and excision of vaginal metastases and then received dactinomycin and etoposide without response. She then received high-dose methotrexate and folinic acid without response before being referred for ifosfamide therapy. At the outset of treatment with this agent, the hCG level was 342 mIU/ml and there was a 3.0 × 3.0 cm nodule in the right lower lung field on radiograph.

She received a total of five courses of ifosfamide and mesna. The starting dose of ifosfamide was 1.2 gm/m², but this was escalated to 1.5 gm/m² because of lack of hematologic toxicity. With treatment every 4 weeks, this patient had a measurable partial response in the pulmonary nodule and a titer response to 32 mIU/ml before brain metastases developed. She died 6 months later (Fig. 1).

Case 3. An 18-year-old black woman, para 2-1-1, underwent suction curettage in December 1984 for a hydatidiform mole. Before evacuation, the hCG level was 167,000 mIU/ml. Within 10 weeks the hCG level was undetectable; however, in January 1986 the hCG value was 52 mIU/ml. Physical and ultrasonographic examination of the pelvis, as well as abdominal and cranial computerized tomograms, all gave normal results. A curettage yielded secretory endometrium, and the uterine cavity was sounded to 7.5 cm. She received four courses of chemotherapy with methotrexate with little drop in the hCG level, so dactinomycin and chlorambucil were added. Seven courses of MAC were administered, and in August 1986 a 1 cm nodule was noted on a chest radiograph. A fine-needle aspiration confirmed the diagnosis of metastatic choriocarcinoma, but the patient refused surgical resection of this mass and was transiently lost to follow-up.

In 1987 she received four courses of chemotherapy with bleomycin, etoposide, and cisplatin, and the disease was believed to be in remission. On June 10, 1988, she presented with an hCG level of 141,000 mIU/ml and a 3 cm left breast mass. Another curettage was performed and scanty tissue was obtained. On Aug. 8, 1988, the breast mass was 7 cm in diameter, and a biopsy confirmed the presence of metastatic choriocarcinoma. The hCG level was then 926,130 mIU/ML, and a large pleural effusion and multiple pulmonary nodules were present on a chest radiograph. She was referred to Indiana University Medical Center. Computerized tomography of the head demonstrated five discrete metastatic lesions that were enhanced with contrast. There was a right-to-left shift of the midline structures. On abdominal tomography, there were bilateral renal metastases, a nonfunctioning right kidney, and no apparent pelvic disease. She was treated with dexamethasone, dilantin, and 3000 cGy whole-brain radiotherapy in 300 cGY fractions with cobalt 60. Chemotherapy was initiated. She received 14 courses of EMACO (Fig. 2), consisting of etoposide 100 mg/m², dactinomycin 500 μg, vincristine 1.0 mg/m², and cyclophosphamide 600 mg/m². Methotrexate 100 mg/m² as an intravenous bolus and 200 mg/m² infused over 12 hours was given with intramuscular folinic acid rescue 15 mg every 12 hours times four. The hCG level decreased but began to rise in June 1989. After the rise was confirmed, therapy with etoposide, ifosfamide, and cisplatin was started. She received etoposide 75 mg/m², ifosfamide 1.2 gm/m², and cisplatin 30 mg/m² daily for 2 days

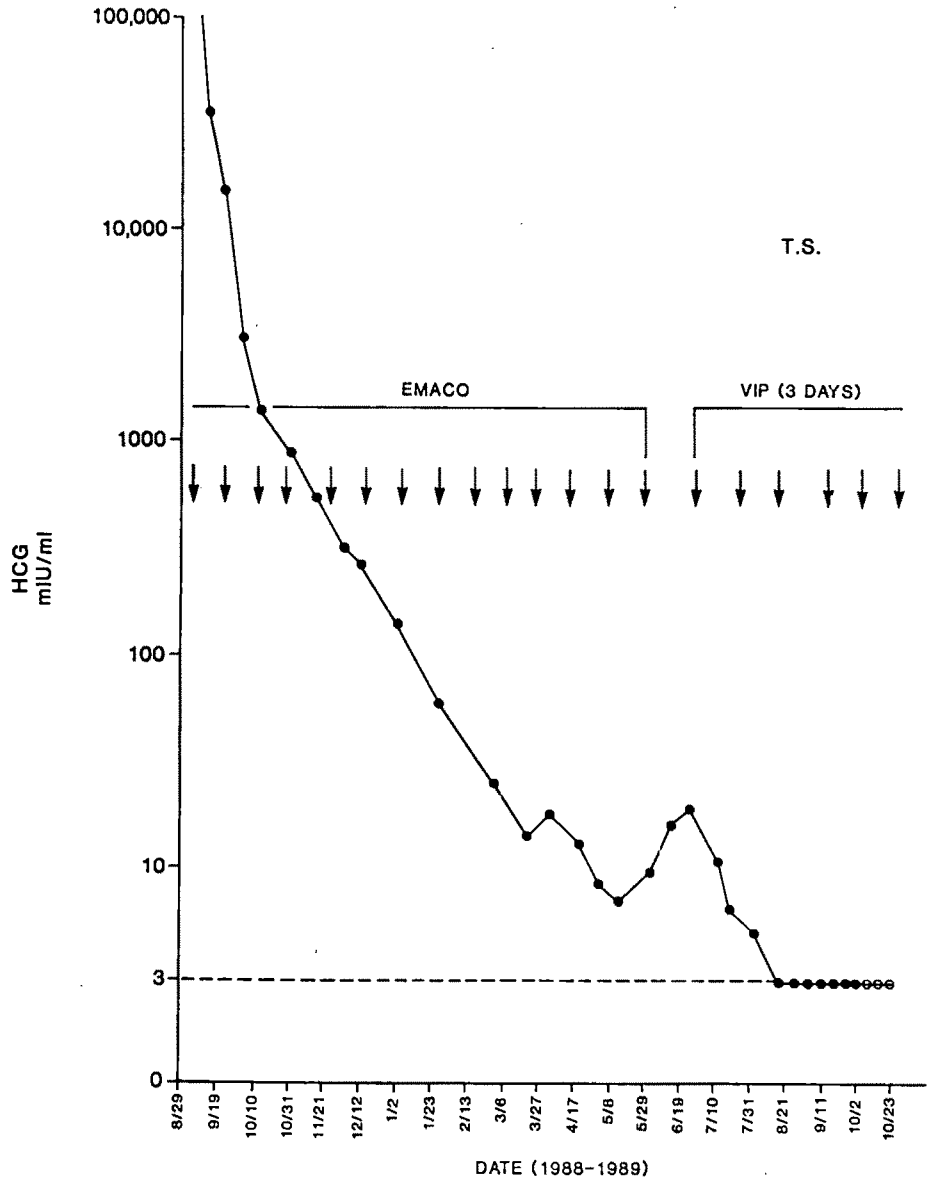


Fig. 2. Permanent remission induced with repeated 3-day courses of VIP therapy in EMACO-resistant patient (case 3).

during the first course and for 3 days during subsequent courses. Mesna was given in a 120 mg/m² intravenous bolus followed by 1.2 gm/m² intravenous infusion. Courses were repeated as often as counts permitted, about every 21 days. The hCG level was undetectable after the third course, and four additional courses were given. She was hospitalized twice for febrile neutropenia, which responded to parenteral antibiotics. The hCG values have remained <3.0 for 34 months since the first undetectable level and she is without symptoms.

Case 4. A 52-year-old white woman was referred to Duke University Medical Center in March 1988. She had had a term vaginal delivery 29 years before admission and a spontaneous abortion in 1982. She underwent an abdominal hysterectomy for bleeding in 1983 with no cancer seen histologically. She presented with a thyroid mass, numerous pulmonary metastases, and mediastinal lymphadenopathy. The initial hCG level was 30,000 mIU/ml. An open biopsy of the thyroid mass demonstrated choriocarcinoma of unknown origin, and abdominal computerized tomograms revealed multicystic enlargement of the ovaries. Shortly after admission, she underwent bilateral salpingo-oophorectomy; theca-lutein cysts were confirmed histologically. She received seven cycles of EMACO chemotherapy ending in June 1988. An hCG nadir of 20 mIU/ml was attained before a rise into the 70 to 100 mIU/ml range. She was treated with cisplatin 20 mg/m² and etoposide 100 mg/m² intravenously daily times five. Remission was attained after the first course of therapy, and she received three additional cycles. In

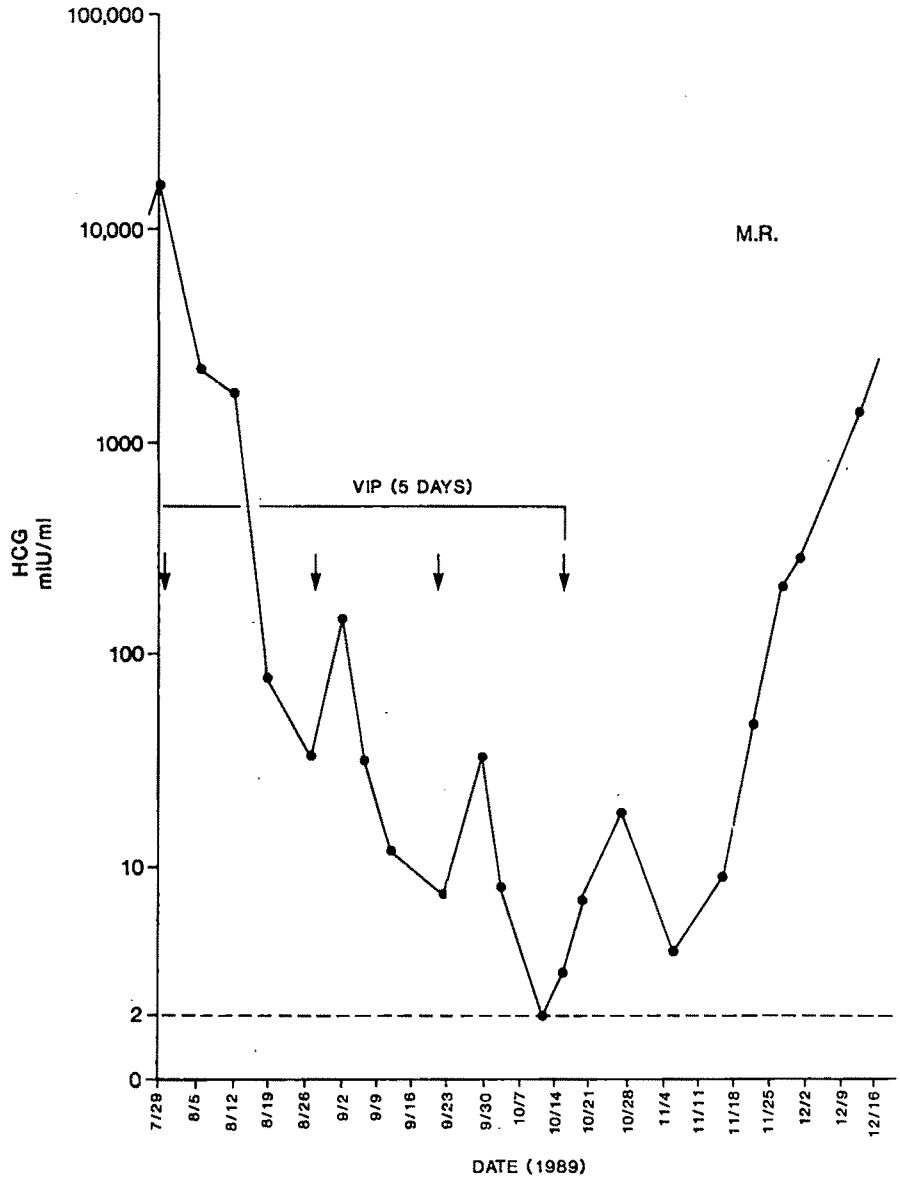


Fig. 3. Transient remission induced with 5-day courses of VIP in case 4. Myelotoxicity precluded rapid recycling of VIP.

October 1988 the hCG level again became elevated into the 70 to 100 mIU/ml range, and two additional cycles of cisplatin and etoposide were given. Bleomycin was added concurrently. The nadir attained after this treatment was 15 mIU/ml. Etoposide, dactinomycin (500 μg intravenous per day), and cyclophosphamide (200 mg/m²) were given for 5 days. She experienced neutropenia and sepsis, and there was no additional fall in hCG response. Two more courses of etoposide as a single agent were given. New pulmonary nodules developed, and the hCG level rose to 134 to 150 mIU/ml.

She next received three cycles of alternating etoposide, methotrexate, and dactinomycin (EMA) and etoposide-cisplatin, but the hCG level remained above 75 mIU/ml. Chest tomography demonstrated persistent small pulmonary nodules, and she received 3150 cGY to the mediastinum and 1575 cGY to whole-lung fields. An hCG nadir of 734 mIU/ml was attained, but the level quickly rose to 15,000 to 17,000 mIU/ml and radiotherapy was abandoned.

VIP therapy was started on July 29, 1989 (Fig. 3). Etoposide 75 mg, cisplatin 20 mg, and ifosfamide 1.2 gm/m² were given intraveous daily times five. Mesna was given as a constant infusion (see case 3). Toxicity of the first course included an increase in creatinine level from 1.6 to 2.3 mg/dl, a nadir hematocrit of 22%, neutropenia of 200 cells/mm³, and thrombocytopenia of 15,000 cells/mm³. Although each of the four courses of VIP was associated with similar toxicity, negative

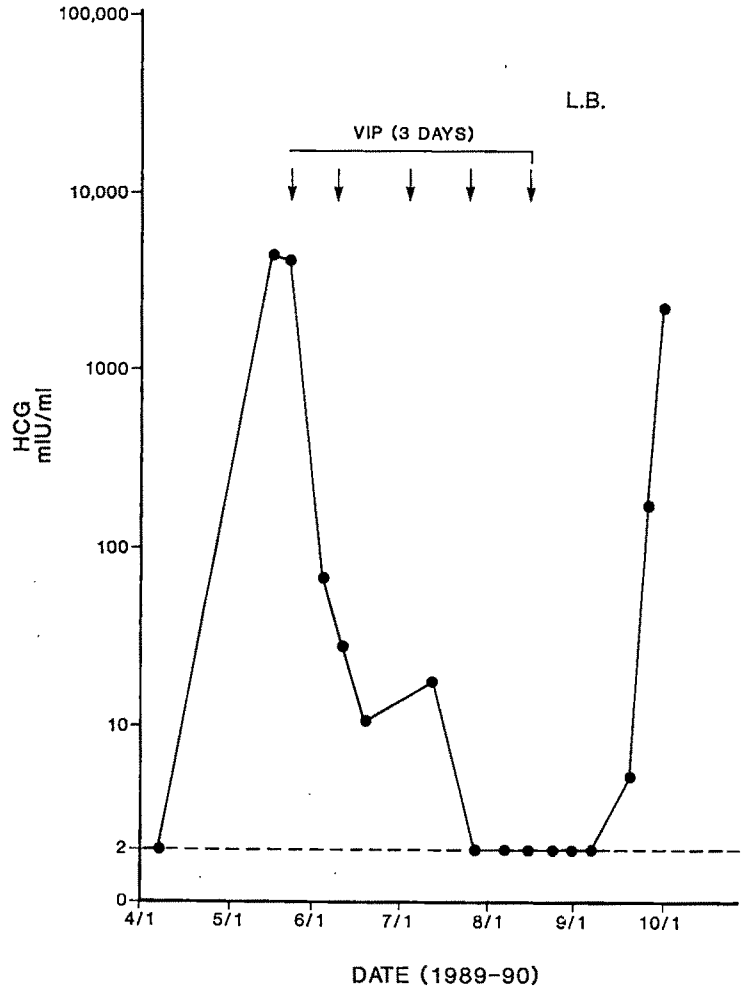


Fig. 4. Remission induced with 3-day courses of VIP in case 5. Relapse followed discontinuation of therapy after only two consolidation cycles.

hCG levels were attained after the third course of therapy. Because of neutropenia, treatment could not be given more frequently than every 24 days. She refused further chemotherapy after a rise in hCG level during the fourth course of treatment and died of respiratory failure in December 1989.

Case 5. A 38-year-old white woman, para 1-0-0-1, underwent evacuation of a hydatidiform mole in 1986. She was treated with two cycles of methotrexate and folinic acid when hCG levels plateaued after the suction procedure. In July 1989 the patient apparently had a missed abortion; curettage and laparoscopy were performed and no pregnancy tissue was demonstrated. In October 1989 abdominal hysterectomy and bilateral salpingo-oophorectomy were performed because of a persistent hCG value in the 600 mIU/ml range. No evidence of choriocarcinoma of ovarian abnormality was identified. The hCG level rose to the 1000 mIU/ml range in spite of two further courses of methotrexate and folinic acid. Five courses of EMACO therapy were given starting in November 1989. By December 1989 hCG levels were undetectable. The last EMACO was given on Feb. 1, 1990. In April 1990 the hCG level was normal, but on May 14, 1990, it had risen to 4400 mIU/ml. She was admitted to Duke University Medical Center where physical examination findings and hematologic parameters were normal. The quantitative hCG value ws 4200 mIU/ml, and computerized tomograms of the brain, chest, abdomen, and pelvis gave normal results except for the absence of gynecologic organs. Cisplatin 30 mg/m^2, ifosfamide-mesna 1.2 gm/m^2, and etoposide 100 mg/m^2 were given daily for 3 days (Fig. 4). The granulocyte count fell to 1200/mm^3, the platelet count to 96,000/mm^3, and the hematocrit to 28%. She experienced nausea and vomiting, which were treated with transdermal scopolamine and lorazepam. The hCG level fell to 68.5 mIU/ml 17 days after the chemotherapy was started. She received a second course of VIP chemotherapy while hospitalized and three additional courses at the referring in-

stitution. The hCG value was <5 mIU/ml at the beginning of the fourth course of chemotherapy. She experienced Gynecologic Oncology Group grade 3 nausea and vomiting in the last three cycles of therapy, as well as grade 3 myelosuppression, leading to a 20% reduction in the doses of etoposide and ifosfamide. The serum creatinine value was unaffected by therapy. Because of gastrointestinal toxicity, this patient declined therapy after the second consolidation course. After 2 months in remission, the hCG level once again was elevated to 5.6 mIU/ml and then 2200 mIU/ml by Oct. 2, 1990. Chemotherapy with etoposide, dactinomycin, methotrexate, and cisplatin was introduced, but the patient ultimately died.

Comment

The aggressive use of multidrug chemotherapy in patients with metastatic gestational trophoblastic disease having a poor prognosis has resulted in permanent remission rates of about 80%. Li et al.[5] introduced the use of "triple" therapy (methotrexate, dactinomycin, and chlorambucil) in patients with testicular cancer in 1960. Hammond et al.[6] demonstrated the advantage of this combination when compared with single-drug therapy at the time of diagnosis in patients with high-risk metastatic disease in 1973.

Bagshawe[7] advocated the use of a seven-drug, 9-day combination called CHAMOMA, and this combination was modified slightly by Surwit et al.[8] when it was introduced in this country. The Gynecologic Oncology Group[1] found that a modified CHAMOMA combination was significantly more toxic and perhaps less effective ($p > 0.05$) than MAC in a prospective randomized two-arm multiinstitution study.

When the activity of a new drug can be demonstrated in patients with chemotherapy-resistant tumors, this agent may be incorporated into a new first-line combination. The alternating, non-cross-resistant (EMA-CO) combination introduced by Bagshawe[2] in 1984 included etoposide because of its marked activity in both previously treated and untreated patients. The therapeutic value and low toxicity of this now popular combination have been corroborated by others.[9]

In 1979, Newlands and Bagshawe[10] reported that cisplatin, either alone or in combination with methotrexate and vincristine, produced responses in patients with refractory trophoblastic disease. Einhorn[11] found that the combination of cisplatin, bleomycin, and vinblastine was highly active in malignant testicular germ cell tumors. Although the initial reports of Schlaerth et al.[12] and Hainsworth et al.[13] suggested a role for this combination in gestational trophoblastic disease, Gordon et al.[14] and DuBeshter et al.[15] failed to achieve sustained remissions with this combination in the majority of patients.

Etoposide and cisplatin were combined with or without dactinomycin in 22 patients reported on by Theodore et al.[16] Long-term remission was induced in all eight patients receiving this regimen without prior therapy and in 11 of 14 patients (78%) with disease refractory to the initial chemotherapy regimens.

The Gynecologic Oncology Group has exhaustively studied ifosfamide as a single agent in patients with gynecologic malignancies. Response rates of 20% and 11.1% have been observed in previously treated ovarian epithelial cancer and squamous carcinoma of the cervix. Uterine sarcomas are very sensitive to this agent; 14.3% of untreated leiomyosarcomas and 30.7% of mixed müllerian tumors responded to therapy.[17]

When the response to ifosfamide described in case 2 was observed, it seemed logical to combine this drug with cisplatin and etoposide in subsequent cases. Loehrer et al.[4] demonstrated the efficacy of this three-drug combination in refractory testicular cancer and initiated a phase III study comparing VIP with cisplatin, etoposide, and bleomycin in patients with untreated testicular germ cell neoplasms. Because of the extent of prior drug exposure, the patient described in case 3 was given just 2 days and then 3 days of VIP, normally a 5-day regimen. The 5-day administration of VIP proved to be prohibitively toxic in untreated patients with small-cell carcinoma of the lung, but this combination could be given safely when the dose was reduced by 20% and given over 4 days.[18] The 3-day course of administration allowed relatively rapid recycling of therapy in case 3 whereas administration of the full 5-day course resulted in toxicity-related treatment delays, which may have allowed "escape" of the tumor in case 4. Case 5 underscores the need to continue therapy for more than one or two "maintenance" courses before the first negative hCG value.[19]

For those few patients with trophoblastic disease resistant to regimens such as MAC and EMACO, further evaluation of ifosfamide-containing regimens seems warranted; earlier use of this agent would also be of interest.

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Circulating concentrations of fetal fibronectin do not reflect reduced trophoblastic invasion in preeclamptic pregnancies

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This prospective, nested, case-control study investigated whether maternal plasma fetal fibronectin reflects reduced trophoblastic invasion at 16 to 20 weeks in women who later have preeclampsia. Concentrations of fetal fibronectin were 8.7 ± 2.6 μg/ml in women with preeclampsia and 8.1 ± 2.5 μg/ml in matched controls ($p > 0.5$). These results are discussed. (AM J OBSTET GYNECOL 1992;167: 496-7.)

Key words: Fetal fibronectin, preeclampsia, implantation, trophoblastic invasion

The cause of preeclampsia remains unknown, but there is evidence that impaired trophoblastic invasion into maternal spiral arterioles plays an important pathogenetic role.[1] This incomplete remodeling of the spiral arteriolar wall occurs at 16 to 20 weeks' gestation, as a result of the failure of the second wave of trophoblastic invasion. Invading cytotrophoblasts in the decidua are surrounded by extracellular matrix, which stains heavily for fetal fibronectin.[2] During pregnancy fetal fibronectin, presumably of trophoblastic origin, can be detected in maternal plasma in concentrations that increase with the duration of pregnancy.[2] We hypothesized that circulating concentrations of fetal fibronectin would be reduced at 16 to 20 weeks' gestation in women who subsequently have preeclampsia, when compared with matched controls who subsequently carry a normal pregnancy.

Material and methods

A prospective, nested, case-control study design was used. Blood collected in 5 mmol/L ethylene diaminetetraacetic acid was drawn at 16 to 20 weeks' gestation and stored as plasma at −70° C. After pregnancy women were identified as preeclamptic if they met strict criteria, namely, late-pregnancy hypertension (an absolute blood pressure ≥140/90 mm Hg, or a rise of ≥30 mm Hg systolic or ≥15 mm Hg diastolic over values in the first 20 weeks), proteinuria (≥1+ in a catheterized specimen or ≥2+ in a voided specimen), and hyperuricemia (serum uric acid ≥1 SD above normal for gestational age) in a nulliparous woman whose clinical and laboratory findings resolved by 12 weeks post partum. Controls, also nulliparous, were matched for maternal age, gestational age, and race. All patients were delivered at term. This study was approved by the university's Committee on Human Research.

Fetal fibronectin was measured in triplicate by a sensitive and specific enzyme-linked immunosorbent assay that used the FDC-6 antibody (ROMCheck, Adeza Biomedical, Sunnyvale, Calif.). This antibody recognizes a glycosylated hexapeptide located in the IIICS region that is unique to the fetal isoform of fibronectin.

Results were compared with paired, two-tailed t tests and are reported as means ± SD, with a $p < 0.05$ considered significant.

Table I. Maternal and neonatal clinical characteristics and plasma fetal fibronectin concentrations

	Preeclamptic	*Control*	*p Value*
No.	10	10	
Age (yr)	24.1 ± 4.6	23.8 ± 4.8	NS
Gestational age (wk)	17.2 ± 1.1	17.2 ± 1.2	NS
Maternal weight (kg)	63.0 ± 13.4	59.0 ± 10.6	NS
Neonatal weight (kg)	3.17 ± 0.72	3.43 ± 0.39	NS
Maternal serum α-fetoprotein (ng/ml)	54.4 ± 30.0	55.3 ± 39.9	NS
Plasma fetal fibronectin (μg/ml)	8.7 ± 2.6	8.1 ± 2.5	NS

NS, Not significant.

Results

There were no significant differences between preeclamptic and matched control patients in maternal

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6/1/38691

age, gestational age, maternal weight, infant weight, maternal serum α-fetoprotein levels, or fetal fibronectin concentrations (Table I). The values for all comparisons were $p > 0.3$.

Comment

Immunohistochemical studies demonstrate the presence of fetal fibronectin in the extracellular matrix of the decidua, amnion, and chorion but not in the cytoplasm of trophoblastic or decidual cells.[2] When designing this study, we posited that fetal fibronectin enters the maternal circulation as a consequence of trophoblastic invasion of maternal spiral arteriolar walls. We further hypothesized that the degree of invasion, which should be decreased in pregnancies complicated by preeclampsia, would correlate roughly with maternal plasma fetal fibronectin levels.

There are several possible explanations for the absence of such a correlation in this carefully matched cohort of preeclamptic and control patients. First, in spite of its high molecular weight, the entry of fetal fibronectin into the maternal circulation may not require gross invasion of the spiral arteriolar wall and thus may not correlate well with the characteristic histologic findings in the placental bed of preeclamptic patients. It is possible that in all pregnancies there is sufficient disruption of the spiral arteriolar basement membrane to allow the passage of fetal fibronectin.

Second, extravillous trophoblast may not be the primary source of maternal plasma fetal fibronectin. For example, fetal hepatocytes and renal tubular cells contain fetal fibronectin,[2] and the transplacental transfer of this protein from fetal blood or amniotic fluid as with α-fetoprotein, is possible. However, we found a poor correlation between fetal fibronectin levels and α-fetoprotein levels in these patients ($r = 0.257$, data not shown).

Third, data suggesting reduced trophoblastic invasion in preeclampsia are based on placental bed biopsies obtained after the development of clinical disease. Although these findings have been interpreted as a failure of normal invasion early in pregnancy, another interpretation is that initial invasion is normal in preeclamptic women and regresses later in gestation.

Further studies are required to elucidate the possible role of fetal fibronectin in the pathogenesis of preeclampsia.

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Resident research in obstetrics and gynecology: Development of a program with comparison to a national survey of residency programs

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OBJECTIVE: The objective of this report is to describe our recently established resident research program and to compare this program with those of other obstetrics and gynecology residency training programs in the United States.

STUDY DESIGN: The components of our program are described. Data for comparison from other programs were obtained from questionnaires, phone follow-up and the *Directory of Obstetrics and Gynecology Residency Programs* of the Council on Resident Education in Obstetrics and Gynecology.

RESULTS: We initiated a residency research program in 1987 with acceptable projects defined, a time for completion of manuscripts established, and a Resident Research Day organized with presentation of articles, critique by a guest speaker, and an awards banquet. Nationally, <60% of residency programs have research requirements. Of the 208 programs responding to the written questionnaire 86% of programs associated with a university required research compared with 10% of community-based residency programs.

CONCLUSION: While more than a third of residency programs in obstetrics and gynecology do not have resident research programs, the trend is for programs to establish research as a required portion of training. An outline of a recently established program is provided. (AM J OBSTET GYNECOL 1992;167: 498-502.)

Key words: Resident research, resident training, program accreditation, obstetrics and gynecology residency programs

Many residency programs in obstetrics and gynecology require the involvement of their residents in research, but the actual number of programs providing research training and the nature of these programs are not available. Resident research activities can encourage critical thinking, stimulate self-learning, and promote exploration and assessment of career opportunities, as well as provide significant contributions to the literature.[1-4] Research projects provide experience in the review and evaluation of scientific literature, training in the conduct of research projects and preparation of manuscripts, and opportunities for training and experience in public speaking. Because our department recognized a deficiency in these opportunities for our residents, we formally initiated resident research in 1987 and a Resident Research Day in 1988.[5] Guidelines and a timetable were established to assist the residents in planning and completing projects and in preparing subsequent formal presentations. To compare our residency research training program with other programs, we initiated a national survey of obstetrics and gynecology programs in May 1990.

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Material and methods

Development of a resident research program. A plan for ongoing resident research projects culminating in an annual Resident Research Day was initiated at our institution in 1987. The purposes of establishing resident research projects were as follows: (1) to teach residents to critically review the medical literature, to organize and conduct a research project with an appropriate understanding of all aspects of experimental design and statistical analysis, and to prepare a manuscript; (2) to teach residents to organize a formal presentation including the preparation of visual aids and to present formally to an audience of their peers; (3) to promote research, formal presentations, and the publication of manuscripts as essential components of an academic residency training program and of a career in obstetrics and gynecology.

Guidelines for annual resident research projects were established, including definitions of acceptable projects, a time line for completion of each phase of each project, faculty supervision of projects, a faculty coordinator

Table I. Response to resident research survey of 280 obstetrics and gynecology residency programs

	No.	*% of responders*	*% of all programs*
Written responders			
"Yes" responses	142	68	51
"No" responses	66	32	23
Subtotal	208	100	74
Phone responders			
"Yes" responses	6	26	2
"No" responses	17	74	6
Subtotal	23	100	8
TOTAL RESPONDING	231		82

Table II. Relationship of program size and university connection to the proportion of obstetrics and gynecology residency programs requiring research

Total No. of residency positions	*Programs associated with a university*		*Community-based programs*	
	No.	*% with Research*	*No.*	*% with Research*
≤10	33	82	15	13
11-20	81	86	31	10
≥21	45	89	3	0
TOTAL*	159	86	49	10

*Of 208 programs responding to written questionnaire, proportions requiring research are significantly different ($p < 0.0001$) between programs associated with a university and those that are community based.

for the overall Resident Research Day, and formal presentation of projects at the annual Resident Research Day. A format was proposed for printing of all manuscripts in a booklet, presentation of articles to the faculty and resident staff, critique of presentations by a guest faculty, judging of research projects and formal presentations, presentation of awards at an annual Resident Research Day Banquet, and submission of manuscripts to scientific journals for formal publication. To compare our resident research program with others, a national survey of all accredited obstetrics and gynecology programs was conducted.

Resident research questionnaire. A questionnaire was sent to 280 accredited obstetrics and gynecology programs. Each program director was asked, "Does your residency program have a formal resident research program that is required for completion of the residency?" If the response was "yes," then the year in which the program was initiated, the number of research projects required during the residency, and specific questions about the research were asked, including criteria for acceptable projects, research guidance available to residents, and means of presentation of projects made available to residents. If the response was "no," then the percentage of residents electively doing research, the reasons for not requiring resident research, and whether a formal program was contemplated within the next 2 years were surveyed.

Results

Resident research questionnaire: "Yes" responders. Of the 280 questionnaires that were mailed, 208 were returned, for a written response rate of 74% (Table I). Seventy-two of the 280 programs surveyed did not return the questionnaire. Twenty-five of these nonresponders to the written questionnaire were randomly chosen for phone follow-up and were asked if they require residency research for the completion of the residency program. Twenty-three of 25 programs were reached by phone, and their response to the question of having a research requirement is shown in Table I. Programs that require residency research were more inclined to respond to the written questionnaire than were programs that do not require research. Sixty-eight percent of the 208 programs that returned the questionnaire reported that research is required and 26% of the 23 programs polled by phone reported that residency research is a requirement. The difference was statistically significant by χ^2 analysis ($p < 0.0001$). To verify the written responses, we randomly chose for phone follow-up 20 residency programs that had responded "yes" on the written questionnaire. Nineteen of these 20 confirmed that resident research is a requirement for the completion of the residency. One program indicated that resident research was a requirement on the written form but did not confirm that research was required to complete residency on phone follow-up for a 5% error rate. Of the total 231 respondents, 148 (64%) require their residents to complete a structured research program whereas 83 (36%) do not. Table II shows the relationship of program size as measured by the total number of approved residency positions and of university connections to the proportion of programs requiring research.

Format for resident research. The design and requirements of our residency research training program were compared with those of other programs. When our program was initiated in 1987, a Director of Resident Research was appointed to coordinate resident research activities and Resident Research Day. Each second-, third-, and fourth-year resident is required to complete a yearly project under the supervision and with the approval of a faculty advisor. To complete each project satisfactorily, a manuscript of the work, written by the resident and approved by the faculty advisor, is formally presented by the resident on Resident Research Day. Thus, for completion of our residency research requirements, a resident must complete three projects

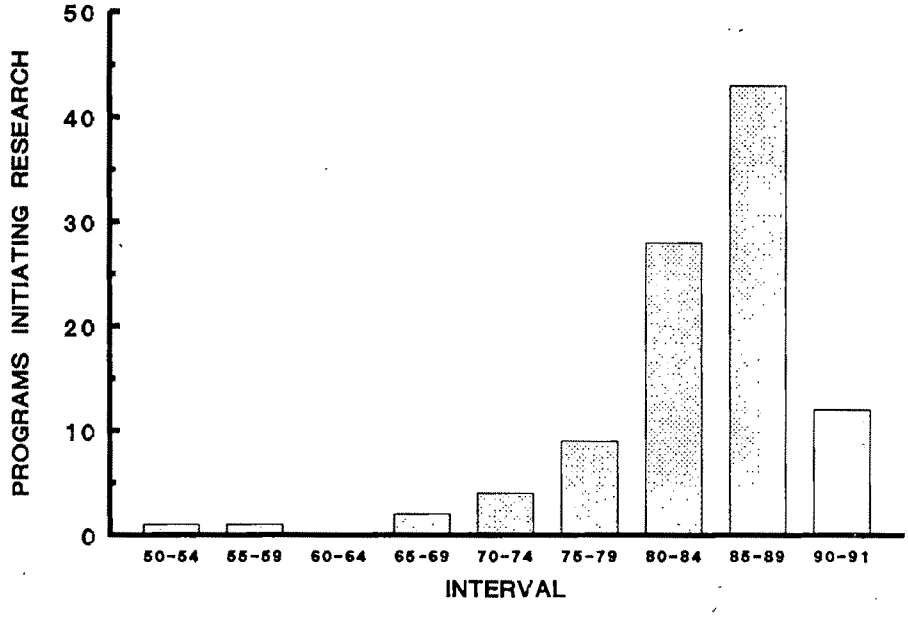


Fig. 1. Year resident research initiated in obstetrics and gynecology program requiring resident research.

culminating in written manuscripts with each formally presented. This format was compared to the responses from the 142 programs with a research requirement that completed the written questionnaire. Fig. 1 shows the year in which residency programs initiated formalized research requirements. Whereas we require the completion of three projects, 79% of the programs require only one project during the entire residency. Twelve percent require two projects, 8% require three projects, and only 1% require yearly projects.

Our residency research program outlines specific guidelines for acceptable projects. The annual projects may consist of basic or clinical research, including prospective studies, retrospective studies, and case studies. Each resident is to complete at least one prospective study during the residency, which should be planned during the first or second year to ensure completion by spring of the fourth year of residency. The remaining two projects can include a case study or retrospective studies. Only one case study or case series is allowed. Our guidelines are similar to those of other residency programs that require residency research in that the majority require retrospective or prospective studies (Fig. 2).

Resident research instruction. Realizing that resident research adds to the demands already placed on our residents but recognizing the benefits to the residents, we take several measures to assist them. A faculty adviser supervises each project throughout its many phases, from developing the idea to practicing the presentation. We also establish a 1-month resident research rotation with the Research Director of the Department of Obstetrics and Gynecology. The residents receive instruction in research philosophy, literature review, experimental design, research methods, and statistics. Additionally, each month a 1-hour research conference is conducted for the residents and staff with topics focusing on the mechanics of developing a research proposal, experimental design, computer use, literature review, record keeping, statistical methods, analysis of results, writing abstracts and manuscripts, and public speaking. Our monthly journal club also supports resident instruction in research by critically evaluating recently published articles and classic articles from the past. Of the 142 responding programs that require residency research, 96% indicated that residents have a faculty advisor for each project although only 48% of these programs have formal instruction for residents on how to conduct research.

Presentations and manuscripts. A formal Resident Research Day was established at our institution as an annual departmental event held each April. The day includes the residents' presentations of their annual research projects, critique of the projects by a guest reviewer, resident awards, and an evening banquet. A booklet of the residents' final manuscripts is prepared for distribution on Resident Research Day. The manuscripts are sent to the guest reviewer at least 1 week in advance. Each resident is allotted 15 minutes to present a retrospective or prospective study or 10 minutes to present a case report or series. The guest reviewer critiques each presentation and a question-and-answer session follows. An Awards Committee, comprising the guest reviewer and four faculty members, is appointed

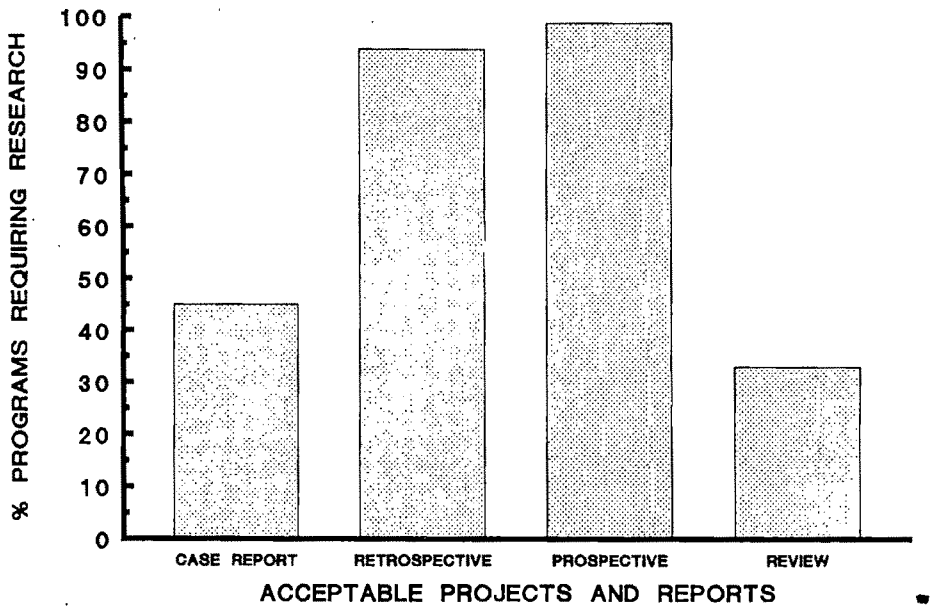


Fig. 2. Acceptable projects and reports for obstetrics and gynecology programs requiring resident research.

by the department chairman to select a "best paper" and a "best presentation." These awards are presented at a banquet the evening of Resident Research Day. Of the 142 programs that require resident research, 94% reported that the resident projects are presented in a formal manner, similar to our annual resident research day. Seventy-seven percent said that residents are required to submit a formal manuscript of their research while for 23% this is not a requirement.

In developing our residency research program, publication of a manuscript was not set as a requirement. We have completed 4 years of residency research, and to date 10 of the projects have been published or have been accepted for publication in the scientific literature and five are currently submitted or are in preparation for submission. In response to the questionnaire, only 15% of the programs that require residency research require that project manuscripts be submitted for publication. We have not made publication in the medical literature a requirement, but we have been very pleased with the quality of the projects presented thus far.

Resident research questionnaire: "No" responders. Sixty-six programs that responded to the written questionnaire said they do not require research and many cited more than one reason for not doing so. The predominant reasons expressed were that the residents were too busy (52%), that the staff was too busy to assist the residents (28%), and that research should not be a requirement during residency training (46%). Of the programs that do not require residents to perform research, most have ≤25% of their residents electively doing research. Of the programs not requiring research, 42% do not contemplate initiating a formal residency research program within the next 2 years, while 43% do plan to initiate one. Fifteen percent did not respond to this question.

Comment

We had a very good response to our questionnaire. Of 280 programs surveyed, 208 returned the questionnaire with 142 of these requiring resident research for completion of training. Of the 72 nonresponders to the written questionnaire, 23 were contacted by phone with 26% of this study group stating that they do require research. If we apply the 26% figure to all 72 nonresponders, then we can estimate that approximately 19 of these programs require research, for a total of 161 (142 + 19) programs (58%) among the entire 280 in the United States. Ninety-five percent of programs confirmed their written response in telephone follow-up. From our survey we found that most resident research programs have been initiated since 1980 and many programs plan to initiate a research requirement for completion of residency training within the next 2 years.

The residency review council and boards require residency programs to integrate research into residency training, but the structure and supervision of this aspect of residency are left up to each residency director. The information obtained from our survey of obstetrics and gynecology residency programs should be of interest to all those participating in residency training. It is noteworthy that programs directly associated with a university or medical school more frequently had a re-

search requirement than did programs that are community based. The size of residency programs did not seem to be related to whether the program required research (Table II). Other aspects of the influence of a research requirement on the nature and academic rank of applicants to programs or the career choices and opportunities of graduating residents were not dealt with in our survey. A survey of candidates applying to programs and of physicians a few years after completing an obstetrics and gynecology residency would be needed to see if a resident research requirement influences program choices by medical students and career choices by graduates. The results of that survey will be important in assessing the impact of research requirements.

Our resident research program was comparable to those of other institutions requiring residents to perform research. We require a greater number of projects than do most programs, but we have also incorporated research time and instruction in residency training. Our director of departmental research plays an active role in assisting residents in projects and research instruction.

Since we initiated this program in 1987, our residents have achieved the Clinical Scholar Award for the best resident research at our hospital 4 years in a row. Two projects have been selected by the Southern Medical Association as Best Resident Presentations at their annual meetings in 1988 and 1989. Two projects have won the Pritchard Prize for Best Resident Paper at the Texas Association of Obstetrics and Gynecology Annual Meetings in 1989 and 1991. Several articles have also been presented at other meetings, including the Society for Perinatal Obstetricians in 1990 and the Central Association of Obstetrians and Gynecologists in 1990.

Many of the program directors surveyed responded that they do not require resident research because they feel the quality of articles would be poor if residents were forced to do research; however, we have not found this to be so at our institution. We have been delighted by our residents' enthusiasm and efforts in planning and conducting their projects and in submitting their manuscripts for publication in journals and for presentation at scientific meetings. Before the initiation of a formal residency research program and Resident Research Day, no research presentations or publications were made by the residents in our department. Residents now routinely discuss research activities, willingly pursue potential research projects, and, when confronted by new or interesting clinical problems, are more likely to search the literature and do so with more ease and confidence. A formal resident research program contributes significantly to the overall education of obstetrics and gynecology residents. At the same time the resident research program will enable compliance with Accreditation Counsel for Graduate Medical Education Guidelines for Residency Programs as evaluated by our residency review committee. Our residents have developed a foundation on which to build and pursue research endeavors, whether in private practice or in academic medicine.

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Nonimmune fetal hydrops caused by bilateral type III congenital cystic adenomatoid malformation of the lung at 17 weeks' gestation

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Type III congenital cystic malformation of the lung with nonimmune hydrops and oligohydramnios was diagnosed at 17 weeks by ultrasonography. Massive fetal cardiac compression with probable associated left- and right-sided failure causing both the oligohydramnios and the ascites, respectively, was thought to be the underlying pathophysiologic mechanism of this unusual clinical presentation. Pathologic examination after termination of the pregnancy confirmed the prenatal diagnosis. (AM J OBSTET GYNECOL 1992;167:503-5.)

Key words: Prenatal ultrasonography, nonimmune hydrops, congenital cystic adenomatoid malformation of the lung

Congenital cystic adenomatoid malformation of the lung is a rare form of congenital lung disease. The cause and heredity are unknown.[1] This hamartomatous lesion resulting from cessation of bronchiolar maturation with an overgrowth of mesenchymal elements and proliferation of polypoid glandular tissue in the absence of normal alveolar differentiation creates the "adenomatoid" histologic appearance. These structures are lined by cuboidal or ciliated columnar epithelium. Congenital cystic adenomatoid malformation has been classified into three types that differ primarily in the size of the cysts along with other histologic and clinical features.[1] Type I lesion has some large cysts and type II has multiple evenly sized cysts, whereas type III consists of a firm bulky mass, uniformly formed by small cysts that usually involve an entire lobe. Type III is the rarest, with bilateral involvement being reported six times previously.[1] We present a case in which bilateral type III congenital cystic adenomatoid malformation of the lung was associated with nonimmune fetal hydrops and oligohydramnios at 17 weeks.

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6/1/38306

Case report

A 28-year-old woman, gravida 3, para 0, aborta 2, was referred to Strong Memorial Hospital, the regional perinatal referral center, at 17 weeks' gestation for consultation because of an increased risk for trisomy 21. Her medical history was unremarkable, and the current gestation was uneventful with ultrasonographic confirmation of dates at 7 weeks. At 17 weeks' gestation, maternal serum α-fetoprotein level was 1.83 multiples of the median (normal) and the serum human chorionic gonadotropin level was elevated at 3.87 multiples of the median (77,533 mIU/ml). She was counseled as to the increased risk of trisomy 21 calculated at 1:228 (vs 1:755 for age only); she elected to undergo genetic amniocentesis. Ultrasonography before this procedure revealed a singleton, vertex-presenting fetus with markedly decreased amniotic fluid. Examination of the fetal chest disclosed an abundant amount of hyperechoic tissue representing the fetal lungs. This condition was bilateral and continuous throughout the chest. Massive compression of the fetal heart was noted with the fetal heart occupying a relatively small portion of the chest (<10%) (Fig. 1, *a*). Although a detailed echocardiographic examination was not technically possible, ultrasonographic evaluation at the four-chamber level revealed two ventricles, an intraventricular septum, two atria, and two atrioventricular valves. A large amount of ascitic fluid was noted in the fetal abdomen with an increased abdominal circumference consistent with 25 weeks' gestation (Fig. 1, *b*).

These ultrasonographic findings were interpreted as consistent with bilateral type III congenital cystic adenomatoid malformation of the lung. Because of the poor prognosis associated with the extensive bilateral condition, the parents elected to terminate the preg-

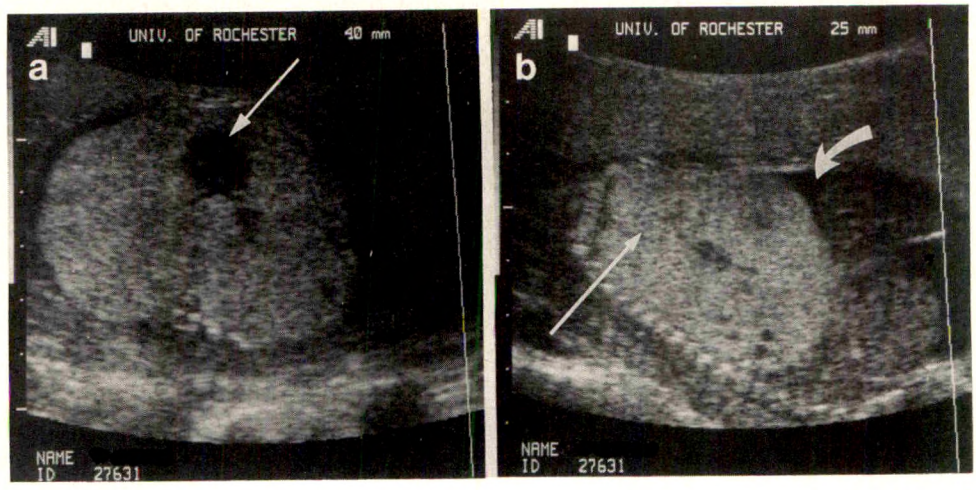


Fig. 1. Prenatal ultrasonographic views at 17 weeks' gestation. **a,** Transverse view of fetal chest. *Arrow,* Fetal heart compressed by abundant lung tissue. **b,** Longitudinal view of fetal chest and abdomen. *Arrow,* Hyperechogenic lung tissue; *curved arrow,* ascites.

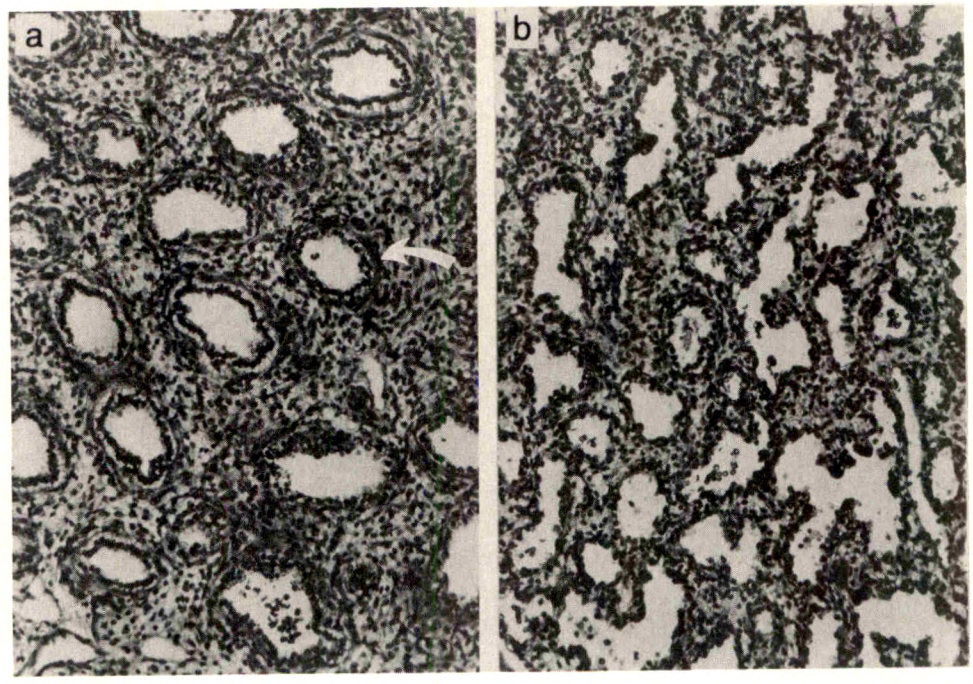


Fig. 2. a, Histopathologic section of lung tissue of described fetus. Note decreased terminal air spaces surrounded by increased smooth muscle *(arrow)* consistent with type III congenital cystic adenomatoid malformation of lung. (Original magnification ×20.) **b,** Normal lung of gestational age–matched control. (Original magnification ×20.)

nancy without an amniocentesis. At the parents' request, an evacuation was performed while the patient was under general anesthesia. Pathologic examination revealed abnormally large fetal lungs (weighing 25 gm, >97.5th percentile for gestational age). Multiple grooves were noted on the outer aspects of the fetal lungs and were identified as rib impressions caused by increased intrathoracic pressure. Histopathologic studies throughout four random sections of fetal lung disclosed notably decreased terminal air spaces and increased smooth muscle, consistent with bilateral type III congenital cystic adenomatoid malformation of the lungs (Fig. 2, *a*). Histopathologic studies of a gestational age–matched control are shown for comparison (Fig.

2, *b*). Fetal kidneys and liver were normal. The fetal head was traumatized during the evacuation and thus was not available for evaluation. The placenta and umbilical cord were normal. Karyotyping was obtained at the time of the evacuation and subsequently revealed a 46,XX karyotype.

Comment

Neonatal outcome of fetuses with congenital cystic adenomatoid malformation of the lung depends on the type of lesion and, as with all intrathoracic space–occupying lesions, on the presence or absence of pulmonary hypoplasia (dependent on the extent of pulmonary involvement). Although the prognosis for a unilateral type I lesion is excellent after resection, type II lesions are associated with a high incidence of structural and chromosomal abnormalities and an overall poor prognosis. Fetuses with type III lesions have a poor outcome on the basis of pulmonary hypoplasia or hydrops.

Spontaneous improvement in utero has been reported in cases of fetuses with unilateral involvement of both type I and type II lesions. Approximately two thirds of fetuses with antenatal diagnosis of this condition will have hydropic changes with either polyhydramnios or oligohydramnios.[2] Although polyhydramnios noted to be associated with this anomaly is attributed to esophageal compression with subsequent impaired fetal swallowing, it is interesting to note similar cases (as ours) where nonimmune fetal hydrops existed with severe oligohydramnios. Our case and three previously published cases of fetuses with bilateral type III congenital cystic adenomatoid malformation of the lung[1,2] suggest that, at least in these cases, combined right- and left-sided cardiac failure caused by cardiac compression may be the underlying pathophysiologic mechanism behind the associated nonimmune hydrops and oligohydramnios.

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Case Reports

Pregnancy complicated by chronic cardiomyopathy and an automatic implantable cardioverter defibrillator

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The dismal prognosis of dilated cardiomyopathy and its resulting tachyarrhythmias has recently been improved by technical advances such as the automatic implantable cardioverter defibrillator. We report a successful pregnancy in a patient with chronic cardiomyopathy and malignant tachyarrhythmias necessitating use of this device, with a documented defibrillator discharge and monitored fetal response. (Am J Obstet Gynecol 1992;167:506-7.)

Key words: Cardiomyopathy in pregnancy, cardiac arrhythmias, automatic implantable cardioverter defibrillator

Chronic dilated cardiomyopathy complicated by cardiac arrhythmias has been variously reported to have a 5-year mortality rate of 50% to 60%. Poor prognostic factors include tachyarrhythmias, left ventricular ejection fraction <35%, myocardial ischemia, and left ventricular dilation sufficient to make the cardiothoracic ratio >0.55 on chest x-ray film.[1] Recent advances in antiarrhythmic medications and technical advances, such as the automatic implantable cardioverter defibrillator, have markedly improved long-term survival.[2] The automatic implantable cardioverter defibrillator was introduced in 1980 for the treatment of malignant ventricular arrhythmias unresponsive to antiarrhythmic medications or surgical ablation.[2] It uses two epicardial defibrillating patches and can be programmed for triggering rate criteria (126 to 208 beats/min) and strength of discharge (1 to 40 J). (Because of direct epicardial application, 8 to 30 J is adequate for cardioversion or defibrillation.) The extended life span afforded by the automatic implantable cardioverter defibrillator and other advances has allowed women with cardiomyopathy to consider pregnancy. Thus the effect of pregnancy on cardiac dysfunction and its effects on the fetus must be considered. To our knowledge, this is the first report of a successful pregnancy complicated by dilated cardiomyopathy and an automatic implantable cardioverter defibrillator.

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Case report

This 22-year-old primigravid woman had viral myocarditis in January 1987 that resulted in a severe chronic cardiomyopathy. The initial left ventricular ejection fraction was 15%, and the left ventricular diastolic diameter was 6.4 cm (normal <5.6 cm). After initial recovery tachyarrhythmias occurred, including ventricular fibrillation in October 1989. Electrophysiologic testing indicated that antiarrhythmic medications could not adequately control the arrhythmias. Thus an automatic implantable cardioverter defibrillator (Ventak 1550 AICD, Cardiac Pacemakers, St. Paul, Minn.) was placed in February 1990. At the time of surgery the ejection fraction was 55%, and she could maintain daily activity without symptoms.

She subsequently became pregnant in May 1990, against the advice of her cardiologist. Her medications included digoxin, metoprolol, furosemide, potassium, and subcutaneous heparin. The first two trimesters were without complications. At 28 weeks' gestation she began to have episodic dyspnea and tachycardia that responsed to bed rest; ejection fraction remained 45% to 50%. At 33 weeks' gestation she experienced a tachyarrhythmia that triggered automatic implantable cardioverter defibrillator discharge. A single 30 J shock resulted in conversion to a normal sinus rhythm and a normal rate. She was monitored, and stable vital signs, a normal electrocardiogram, and a reactive fetal heart tracing were seen.

The patient was placed at bed rest, and both she and her fetus underwent extensive surveillance for the final

month of pregnancy because of increasing fatigue, dyspnea, and activity intolerance. Ejection fraction fell to 35% before delivery. At 37 weeks 3 days' gestation spontaneous rupture of membranes occurred, followed by labor. An introducer sheath (cordus) was placed to monitor central venous pressure and to facilitate rapid introduction of a pulmonary artery catheter, should it be needed. Intrathecal narcotics were used for pain management. An uncomplicated labor resulted in delivery of a viable male infant (Apgar scores of 8 and 9 at 1 and 5 minutes, birth weight 3001 gm), with outlet forceps. Central venous pressure monitoring remained stable for 24 hours post partum, with uneventful recovery. By 6 weeks post partum the patient had returned to full daily activity without symptoms, and ejection fraction had returned to 48%. The infant is doing well.

Comment

Viral myocarditis may occur at any age and generally has no long-term sequelae. Dilated cardiomyopathy is an uncommon but severe potential complication. Historically, dilated cardiomyopathy has had a dismal prognosis, with progressive deterioration, congestive failure, and malignant arrhythmias. Recent advances in medical management and invasive technology have allowed some patients to have a relatively normal lifestyle and to consider reproduction.

Because of the historically poor prognosis, there is no body of literature addressing the effects of pregnancy on chronic dilated cardiomyopathy. The pregnancy reported here was complicated by increasing tachyarrhythmias and decreasing ejection fraction (particularly during the third trimester), which resolved rapidly post partum. The physiologic volume expansion of pregnancy is normally associated with a progressive increase in cardiac output, through increased stroke volume, which peaks at term.[3] In this patient with dilated cardiomyopathy the further increase in left ventricular size necessary to accommodate the excess volume resulted in decreased efficiency (reduced ejection fraction) and increased arrhythmogenic potential. Because this patient was relatively compensated before pregnancy, she progressed beyond the initial volume expansion (24 weeks) before having symptoms.

Tachyarrhythmias are a primary cause of sudden death in chronic dilated cardiomyopathy.[2] The increased tachyarrhythmias experienced in this pregnancy included one of sufficient rate and duration to necessitate cardioversion by automatic implantable cardioverter defibrillator.

No adverse effects on the fetus were noted from the worsening maternal cardiac function or from the automatic implantable cardioverter defibrillator discharge for cardioversion.

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Repeated external cephalic version at term

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External version was performed three times in a patient at term because of spontaneous reversion to breech presentation. She was then delivered vaginally. Spontaneous reversion to breech presentation can be managed with additional external cephalic version procedures. (AM J OBSTET GYNECOL 1992;167:508-9.)

Key words: Breech, external cephalic version

External cephalic version has been reintroduced to modern obstetrics as a safe and effective alternative for delivery of the fetus in a breech presentation.[1] In cases in which immediate induction of labor does not follow successful version, there is a risk of 6% to 7% that the fetus will spontaneously return to a breech or transverse lie.[1] We present a case in which external cephalic version was successfully performed three times at term before a successful vaginal delivery occurred. We discuss the management of breech presentation that reverts before delivery.

Case report

A 37-year-old woman, gravida 2, para 1, was seen at 38 weeks' gestation with a breech presentation. The obstetric and medical history was unremarkable. She had a spontaneous vaginal delivery of a 4025 gm infant 4 years previously. During that pregnancy the fetus remained in a breech presentation until the day of delivery, when spontaneous version to cephalic presentation occurred. The current pregnancy course was uneventful. Dating was confirmed by early second-trimester ultrasonography; genetic amniocentesis was normal. A 1-hour glucose screen after 50 gm of glucola resulted in a blood glucose level 143 mg/dl. A 3-hour oral glucose tolerance test gave a normal result (fasting, 79 mg/dl; 1 hour, 151 mg/dl; 2 hours, 129 mg/dl; 3 hours, 116 mg/dl). Complete breech presentation was diagnosed at 38 weeks' gestation. The estimated fetal weight was 3600 gm; the amniotic fluid index was 20 cm. The patient was counseled regarding the options for delivery. She consented to have external cephalic version, which was easily performed with magnesium sulfate tocolysis. An hour later, during continuous external monitoring, the fetus spontaneously reverted to a breech position. After undergoing counseling regarding her options, the patient agreed to undergo another external cephalic version on the next day to be followed by immediate induction of labor. The second external cephalic version was successful. Cervical ripening with prostaglandin E_2 gel and oxytocin induction of labor were initiated. A serial 3-day induction was attempted with oxytocin infusion for a total of 34 hours. There was no cervical change. The patient requested expectant management rather than cesarean delivery for failed induction. The patient declined further induction attempts for 3 weeks. Antepartum testing was done twice weekly and remained reassuring. At 41 weeks' gestation a breech presentation was diagnosed for the third time. The estimated fetal weight by ultrasonography was 3900 gm with adequate amniotic fluid. After receiving further counseling regarding options, the patient requested cesarean delivery for breech presentation if labor ensued, or if delivery had not occurred in 1 week, she would undergo a third external cephalic version followed by induction of labor. At 42 weeks, external cephalic version was successfully done, and ripening with prostaglandin E_2 and oxytocin induction of labor were accomplished. After a 15-hour labor, a 4140 gm infant was delivered vaginally. Apgar score was 9 at both 1 and 5 minutes.

Comment

Breech presentation at term is associated with a cesarean section rate approaching 90%.[1, 2] External cephalic version reduces the number of breech deliveries and therefore decreases the cesarean section rate. The success rate of external cephalic version at term is approximately 75% while the spontaneous version rate at the same gestational age is only 12% to 17%.[1, 2] After a successful external cephalic version, patients are often permitted to await the spontaneous onset of labor. During this waiting period, 7% of the fetuses revert to breech presentation.[1] There are no established guidelines regarding the management of these patients.

Although external cephalic version is associated with small risks, including fetal distress, abruptio placentae, and fetomaternal transfusion, these risks are acceptably infrequent and manageable with careful patient selection, tocolysis during the procedure, gentle technique, and continuous fetal monitoring during and after the procedure.[1, 2]

External cephalic version is routinely offered to patients with breech presentation at term in many hospitals in the United States. In our case the first and

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second versions were done for the patient's benefaction in view of the higher complication rate of cesarean sections. It seemed prudent to induce labor after the second version, because the fetus quickly reverted to a breech position after the first external cephalic version. When induction failed, the patient was offered a cesarean section, but she declined. Because there was no immediate danger to either mother or fetus, the patient's request for expectant management with careful surveillance was honored according to the principle of patient autonomy. This patient had been carefully counseled on multiple occasions by several physicians regarding her options, risks, and benefits, and she made fully informed decisions. The third external cephalic version was done at the patient's request to employ reasonable options to avoid a cesarean delivery or breech vaginal birth.

We believe that patients who require a second version usually should have labor induced. This case suggests that when induction of labor is unsuccessful, expectant management rather than cesarean section is a reasonable option. It demonstrates that a fetus may spontaneously revert to a breech position twice at term and that external cephalic version may be done three times successfully and safely in selected cases to avoid unnecessary cesarean delivery.

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Congenital deficiency of α-fetoprotein

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Although α-fetoprotein may play a role in fetal immune function or in maintenance of osmotic pressure, its exact function is unknown. We report two infants documented to have congenital deficiency of α-fetoprotein. One infant had cord blood levels <0.5 ng/ml. The second infant had a neonatal level of 120 ng/ml, which is about 2% of the usual concentration for a term newborn. These infants document the existence of congenital deficiency of serum α-fetoprotein. Because it is homologous to albumin, congenital deficiency of α-fetoprotein may be analogous to analbuminemia, a benign genetic trait. (AM J OBSTET GYNECOL 1992;167:509-11.)

Key words: α-Fetoprotein, gene regulation, analbuminemia

The exact function of α-fetoprotein (AFP) is currently unknown.[1] AFP has been suggested to play a role in growth regulation, immune function, and maintenance of osmotic pressure in fetal life.[1] We report two infants with congenital deficiency of AFP, both of whom appeared normal at birth, thereby not supporting the three suggested functions of AFP during intrauterine development. Because AFP is homologous to albumin, congenital deficiency may be analogous to analbuminemia, a benign genetic trait.

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Case reports

Patient 1. A 36-year-old woman gravida 3, para 2, woman who had an amniocentesis at 14.5 weeks' gestation for advanced maternal age. The pregnancy was uncomplicated, and the family history was significant only for myopia in several family members and strabismus in a previous child. Ultrasonography revealed no fetal abnormalities. Amniocentesis was performed without complication. The amniotic fluid cells grew slowly. There was no measurable AFP by electroimmunodiffusion or by radioimmunoassay (<0.5 ng/ml). Because the amniotic fluid total protein concentration was low, the specimen was thought to be maternal urine. A repeat amniocentesis was performed at 17 weeks'

gestation. These amniotic fluid cells grew more rapidly and a 46,XX chromosome pattern was found in 20 cells from three primary cultures. There was still no measurable AFP, but the amniotic fluid total protein concentration was within the normal range. A maternal serum AFP level was obtained at 19 weeks' gestation; it showed a concentration of <0.5 IU/ml. This was determined in a more sensitive radioimmunoassay, obtained by changing the conditions of the assay to derive a standard curve from 0.5 ng/ml to 30 ng/ml. The intraassay and interassay coefficient of variation at this low level was <5%. Repeat fetal ultrasonographic studies at 24 and 32 weeks' gestation showed normal interim growth and no evidence of anomalies. A normal, healthy female infant was delivered vaginally at term with a birth weight of 3.8 kg. Cord blood serum AFP levels were undetectable (<0.5 ng/ml). Maternal serum AFP levels at delivery were also undetectable. Serum AFP levels in one older sibling were <0.5 ng/ml and were 1.0 ng/ml in another sibling and in the father. At age 1 year, this infant was doing well with no physical or developmental problems.

Patient 2. Amniocentesis was performed on a 26-year-old woman, gravida 1, para 0, at 21 weeks' gestation because of two previously undetectable maternal serum AFP levels with normal ultrasonography. Amniocyte chromosome analysis showed a 46,XY pattern. The amniotic fluid AFP concentration was <0.5 ng/ml. The amniotic fluid protein concentrations were within the normal range.

A healthy, normal-appearing term infant was delivered. Birth weight was 3.72 kg and Apgar scores were 9 and 9 at 1 and 5 minutes, respectively. The placenta appeared normal. No cord blood was obtained. About 6 to 8 months after delivery, the original heelstick blood spots from the neonatal screening program were retrieved and analyzed by previously published methods.[2] The AFP concentration was 120 ng/ml, which is about 2% of the usual concentration for a term newborn.

Comment

α-Fetoprotein is the major fetal serum protein and has genetic homology with albumin.[1] It is produced in early embryogenesis by the yolk sac and subsequently by the fetal liver, reaching a peak concentration at 12 weeks' gestation.[3] A small amount of AFP is excreted through the fetal kidney into the amniotic fluid, reaching peak concentrations at 13 weeks' gestation.[3] Smaller amounts cross the placenta into the maternal circulation, resulting in peak concentrations at about 28 to 32 weeks' gestation.[3] After delivery there is a rapid drop in AFP concentration, reaching adult concentration of <10 ng/ml by about 8 months of age.[2] The normal adult male and nonpregnant female level has recently been shown to be 3.04 ± 0.19 ng/ml.

The primary function of AFP in fetal life is unknown. On the basis of previous studies, several functions have been hypothesized.[1] These are: (1) osmotic pressure regulation, (2) carrier transport (steroids, lipids, metals, etc.), (3) growth regulation, (4) blood pH regulation, (5) immunoregulation, (6) hematopoiesis, (7) sexual differentiation (sex hormone binding), and (8) antiprotease activity.

The observation of two normal infants with documentation of congenital deficiency of AFP and presumed prenatal deficiency on the basis of maternal serum and amniotic fluid levels suggests that AFP may not have been obligatory for any of the above functions. However, some of these functions (i.e., growth regulation) require only picomolar or nanomolar amounts of AFP to be effective.

The basic defect in AFP production or regulation in our patients is currently unknown. However, the differences in the neonatal levels of AFP in these two infants suggest the possibility of at least two different mechanisms for this defect, namely gene deletion or decreased production. The latter may be related to defects in the two regulatory genes for AFP found in a mouse model, termed *raf* and *rif*. Defects in the *raf* gene have been suggested as a cause of hereditary persistence of AFP, a benign autosomal dominant trait. In mice, defects in the *rif* gene cause decreased induction of AFP after liver damage and are inherited in an autosomal dominant fashion. A putative *rif*-like gene may be involved in one or both of our patients. However, the exact defect will require further molecular studies.

In addition to the genetic and synthetic mechanisms discussed above, other possibilities for the lack of AFP could be considered. These might include the following: (1) the rapid vascular clearance of nonglycosylated AFP isoprotein variants, (2) glucocorticoid hormonal down-regulation of AFP synthesis at the transcriptional level (a premature extinction of AFP gene activity can be induced by the glucocorticoid hormone dexamethasone in the developing mammalian liver), (3) possible presence of antibodies to AFP, and (4) increased presence of a bound (nonimmunoreactive) form of AFP, noncovalently bound to a blood or tissue component, concomitant with a decrease in the free form. Neither of our patients were on corticosteroid medications or were known to be stressed, making the second possibility unlikely. Most of these situations would probably lead to lower, but detectable, AFP levels.

Given the lack of pregnancy, labor, or delivery complications and the absence of any neonatal problems, congenital deficiency of AFP is likely to be a benign trait, analogous to analbuminemia. From a clinical standpoint, congenital deficiency of AFP should be included in the differential diagnosis of low or absent maternal serum or amniotic fluid AFP levels.

We thank Heinrich Schettler, MD, for referral of patient 1.

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Successful pregnancy outcomes after amniotic fluid embolism

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Two patients who survived cardiorespiratory arrest occurring in association with amniotic fluid embolism syndrome were seen during the subsequent pregnancy. Both had uneventful pregnancies and delivered at term without complications. These are the only case reports of pregnancy after amniotic fluid embolism, and they support a model of amniotic fluid embolism involving chemically abnormal amniotic fluid rather than an unusual sensitivity to normal amniotic fluid. (AM J OBSTET GYNECOL 1992;167:511-2.)

Key word: Amniotic fluid embolism

Amniotic fluid embolism syndrome is an often devastating yet incompletely understood condition characterized in its complete form by dyspnea, hypotension, cardiovascular collapse, and coagulopathy. Reported mortality figures approach 80%.[1] Because of the clinical features, pregnancy subsequent to documented amniotic fluid embolism syndrome is uncommon and to date has not been reported. We report here the first recorded cases of successful pregnancy after documented amniotic fluid embolism syndrome.

Case report

Case 1. The patient, a 37-year-old woman, gravida 3, para 1, aborta 1, was referred for consultation. Her previous pregnancy 5 years before was complicated in the active phase of the first stage of labor by amniotic fluid embolism syndrome with cardiopulmonary arrest, disseminated intravascular coagulopathy, and cesarean delivery for fetal distress at term. The amniotic fluid was clear. The patient was one of the study subjects described in a prevoius manuscript detailing the hemodynamic findings of patients with amniotic fluid embolism syndrome.[2]

This patient desired an additional pregnancy and wanted information regarding risk of recurrence for amniotic fluid embolism syndrome and the optimal mode of delivery. No information was available with which to counsel the patient with respect to these questions, because pregnancy after amniotic fluid embolism syndrome had not been previously reported. The patient subsequently conceived, carried the pregnancy to term, and at her request underwent elective repeat cesarean delivery. Mother and infant did well without any clinical or laboratory evidence of recurrent amniotic fluid embolism syndrome.

Case 2. The patient, a 25-year-old woman, gravida 2, para 1, was referred for care at 35 weeks of gestation after relocating from another area. She had undergone primary cesarean section while she was under spinal anesthesia before labor for breech presentation 2 years previously. As the abdomen was being closed, sudden cardiorespiratory arrest occurred with subsequent disseminated intravascular coagulation and adult respiratory distress syndrome. Squamous cells and fetal hair were recovered from her sputum. She was successfully resuscitated.

The patient desired a trial of labor. She entered the

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hospital in spontaneous labor at 39 weeks and underwent oxytocin augmentation for dysfunctional latent-phase contractions. Labor progressed rapidly and she underwent an outlet forceps delivery without complications. Her postpartum course was uneventful.

Comment

Although more data regarding pregnancy outcome subsequent to amniotic fluid embolism syndrome is desirable, these cases represent the only information currently available. In these two patients amniotic fluid embolism syndrome did not recur during either scheduled cesarean delivery or vaginal delivery after oxytocin-augmented labor.

Our understanding of the pathophysiologic characteristics of amniotic fluid embolism syndrome is incomplete. However, in the primate model the infusion of even large amounts of normal autologous amniotic fluid is clearly not pathologic; recently, an abnormal concentration of amniotic fluid leukotrienes has been implicated.[1] This report supports the role of a qualitatively abnormal amniotic fluid, which may be different in a subsequent pregnancy, as opposed to any unusual maternal sensitivity to amniotic fluid per se in the genesis of this condition. Although any conclusions based on two cases must be regarded as tenuous, it appears that on a theoretic basis, supported by these two cases, repeat amniotic fluid embolism syndrome is probably unlikely.

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Hepatotoxicity with the administration of nifedipine for treatment of preterm labor

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Nifedipine, a calcium channel blocker, is becoming increasingly popular as a tocolytic medication. Previous reports of hepatitis associated with nifedipine in the medical literature have been published. We present the first case reported of a patient with preterm labor treated with nifedipine who had development of a concurrent elevation in serum liver enzyme levels. (Am J Obstet Gynecol 1992;167:512-3.)

Key words: Hepatotoxicity, nifedipine, preterm labor

We report a case in which the patient had preterm labor treated with nifedipine and an elevation in serum liver enzyme levels occurred.

Case report

A 25-year-old primigravid white woman with a 30-week twin gestation was admitted to Long Hospital of the University of California Medical Center at San Franciso for management of preterm labor. Twin pregnancy was diagnosed at 8 weeks' gestation after ovulation induction with clomiphene citrate. Her prenatal course was complicated by frequent uterine contractions at 29 weeks, treated with oral terbutaline. Continued contractions with cervical change prompted admission to our hospital for tocolysis with intravenous magnesium sulfate and subcutaneous terbutaline. On the second hospital day indomethacin was added to her tocolytic regimen because of difficulty in controlling the preterm labor but was discontinued 5 days later because of oligohydramnios. At that time the patient was weaned from intravenous magnesium sulfate to the terbutaline pump. On the eighth hospital day, because of increasing uterine contractions with the terbutaline pump alone, nifedipine was added to the tocolytic regimen at a dose of 20 mg every 4 hours. Good tocolysis was achieved. Mildly consistent elevated blood pressures prompted laboratory tests to rule out preeclamp-

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sia. Of significance, aspartate transaminase (AST) was elevated at 141 U/L (normal 7 to 39 U/L); alanine transaminase (ALT) was elevated at 343 U/L (normal 2 to 54 U/L). Complete blood cell count with differential, platelet count, uric acid, total bilirubin, fibrinogen, prothrombin time, and total urinary protein values were all normal. The patient felt well, denying headache, visual changes, abdominal pain, arthralgias, and pruritus. No fever, nausea, or vomiting occurred. Physical examination was unremarkable and revealed no peripheral edema, hepatomegaly, or right upper quadrant tenderness. She had no history of liver disease, alcohol use, substance abuse, or transfusion. The panel of hepatitis tests gave negative results. Blood pressures subsequent to these laboratory values normalized.

Consultation was obtained from the gastrointestinal service. Monitoring of the patient's bilirubin and prothrombin time for signs of hepatic failure was recommended, and the nifedipine was not to be discontinued unless either the bilirubin or the prothrombin time became elevated. Three weeks after admission the preterm labor could not be controlled with the terbutaline pump and nifedipine. Intravenous magnesium sulfate was restarted, and nifedipine was discontinued. The next day, serum aspartate transaminase peaked at 449 U/L and serum alanine transaminase peaked at 845 U/L. Without nifedipine, titers of both transaminases steadily fell until the patient was discharged 5 days later at 34 weeks' gestation; she was receiving only terbutaline pump therapy after again being weaned from intravenous magnesium sulfate. At that time of discharge, aspartate transaminase and alanine transaminase had fallen to 136 and 378 U/L, respectively. Bilirubin levels and prothrombin time remained within the normal range.

The patient was delivered of healthy twins at 36 weeks' gestation, 48 hours after discontinuation of all tocolytics. At the time of delivery, the aspartate transaminase (AST) level was normal at 37 U/L, and at 4 months postpartum the aspartate transaminase level was normal at 20 U/L.

Comment

Drug-induced hepatotoxicity is not uncommon. The clinical implications are variable, ranging from asymptomatic elevations in transaminase levels to fulminant hepatitis with hepatic failure. Diagnosis is one of exclusion and is supported by rapid resolution of signs and symptoms after drug withdrawal. Rechallenging the patient with the substance and subsequent reappearance of toxic manifestations provide further confirmatory evidence.

Two major mechanisms, hypersensitivity and direct toxicity, have been implicated for the development of hepatotoxicity. Hypersensitivity reactions typically appear 1 to 5 weeks after exposure to the agent and are frequently accompanied by fever, rash, and eosinophilia. The reaction is unrelated to dose and is thought to be a secondary effect of antibodies directed against the drug-hapten complex. Direct hepatotoxicity is often dose-related and is mediated by cryptogenic mechanisms. There is an enhanced female susceptibility to hepatic insults in association with drugs that act by this mechanism.

A review of the literature reveals six publications detailing seven case reports of hepatitis as a result of nifedipine use.[1] All were patients taking the drug for indications of hypertension or angina pectoris. The patients described had signs and symptoms associated with allergic hypersensitivity. Conversely, our patient was asymptomatic; the elevation in transaminase levels was found on screening for preeclampsia. It is unclear whether a more classic picture of allergic hypersensitivity would have developed had she been continued on a regimen of nifedipine for a longer period of time.

Our experience with calcium channel blockers for tocolysis is limited. Recently, Feguson et al.[2] reported cardiovascular and metabolic effects of nifedipine in 27 pregnant patients treated for preterm labor. In this study no elevations in maternal serum transaminase levels were reported after 24 hours of treatment with nifedipine. The transaminase values in these patients were not studied on a long-term basis. In a chart review of 12 patients at our hospital treated with nifedipine (1986 to 1991) for preterm labor, 66% were not screened on a periodic basis for liver function abnormalities in spite of prolonged courses of treatment (up to 68 days of nifedipine, average number of days on nifedipine 19.6).

The safety of drugs that cause only a transient rise in transaminase levels is unclear. Because nifedipine has been implicated in frank hepatitis, the current case serves as a reminder of this potential significant side effect associated with its use. It is our contention that baseline and periodic liver function tests are indicated in the patient on a regimen of nifedipine for the treatment of preterm labor.

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Vaginal foreign body extraction by forceps: A case report

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Cases in which foreign bodies have been inserted into the vagina are uncommon but do occur. The technique and use of obstetric forceps for the extraction of an orange as a vaginal foreign body is described in this case report. Tucker-McLane forceps are the forceps of choice for this technique in this case. (AM J OBSTET GYNECOL 1992;167:514-5.)

Key words: Obstetric forceps, vaginal foreign bodies, foreign body extraction

Vaginal foreign bodies are not common but are seen in the practice of obstetrics and gynecology. Foreign objects may be inserted into the vagina as an aid to masturbation, sexual intercourse, or sexual assault. They may be inserted by the patient or a sexual partner. Many different objects have been used, including bones, tampons, billiard balls, thermometers, swizzle sticks, bottles of all types, drinking glasses, salt cellars, television tubes, wooden shoetrees, pencils, vibrators and other sexual devices, and vegetables and fruits of all varieties.[1] The sequelae of intravaginal foreign bodies includes traumatic lesions to the vagina, rectum, urethra or bladder; infection; stenosis; and vesicovaginal, rectovaginal, or urethrovaginal fistulas. Many different techniques have been used in foreign body extraction from the vagina and rectum. Obstetric forceps were used for extraction of a vaginal foreign body in the case presented here.

Case report

The patient, a 25-year-old white woman, gravida 4, para 3-0-0-3, presented with a viable intrauterine pregnancy at 28 weeks 3 days' gestation. At approximately 4 AM on the day of admission the patient and her intoxicated husband had been engaging in sexual activity when he placed an orange at the perineum and subsequently in the vagina. Neither the patient nor her husband could remove the orange. Later the same morning they were seen by the patient's family practitioner, who was likewise unable to remove the orange. The physician then contacted the labor and delivery triage unit at the hospital for referral. On arrival at the hospital the patient was placed on a monitor to evaluate fetal well-being and to rule out any contractions. Screening ultrasonography revealed a viable fetus in a cephalic position at 28 weeks' gestation. Also noted in the longitudinal plane was the rounded vaginal foreign body. On pelvic examination the perineum was minimally ecchymotic, no abrasions or lacerations were noted, and speculum examination revealed an orange in the proximal vagina. Manual extraction was attempted but was abandoned because of discomfort and inability to apply proper traction in the needed plane. Because of the similar size of the orange and a small fetal head at term, obstetric forceps were considered as a possible extraction method. After signing an informed consent, the patient was taken to the delivery room, placed in a dorsal lithotomy position, prepped, and draped as for a vaginal delivery. The patient was given 50 mg of meperidine hydrochloride by slow intravenous push and a pudendal block of 1% lidocaine for discomfort and to relax the perineum. The bladder was drained with a catheter, as is standard for all forceps procedures. Standard flat-blade Tucker-McLane forceps were lubricated copiously with gel and applied to the orange in a fashion similar to a direct occipitoanterior fetal head. Gentle traction was applied, and the orange was extracted without discomfort to the patient. Careful examination of the cervix, vagina, and perineum revealed no abrasions or lacerations. The patient was taken to the recovery room for maternal and fetal monitoring and subsequently discharged home. The orange had a circumference of 25 cm and a diameter of 7.8 cm.

Comment

Because the patient was multiparous, she had a relatively lax perineum, and the foreign body was round and smooth, similar to a fetal head, she was an ideal candidate for this type of foreign body extraction. The Tucker-McLane forceps was selected for the following reasons. First, the shanks of Tucker-McLane forceps are overlapping and thus cause less perineal stretching or trauma than Simpson forceps. This would be especially important in using this technique in a nulliparous patient. An alternate type might be Elliott forceps. Next, the shanks of the Tucker-McLane forceps are extended, making it easier to reach and apply to a higher, more proximal foreign body. As opposed to standard Simpson or Elliott forceps, the Tucker-

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McLane forceps has solid, nonfenestrated blades that help protect the vaginal sidewalls and perineum during extraction.[2] As with all forceps procedures, the bladder should be empty and appropriate analgesia should be used. The pudendal block worked well in this case, but alternatives include regional or general anesthesia. General anesthesia may be more appropriate if there is suspicion of trauma and a more extensive repair may be needed, especially if there is extension into the bladder, rectum, or peritoneum.

In conclusion, this case represents the use of a common instrument of the obstetrician-gynecologist for use in an uncommon situation. This technique can be added to other techniques for vaginal foreign body extraction. In selected cases it may make extraction easier, with less associated morbidity.

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Leiomyomatosis peritonealis disseminata treated with a gonadotropin-releasing hormone agonist

A case report

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To our knowledge, this is the first report of documented growth regression of leiomyomatosis peritonei while the patient was receiving a gonadotropin-releasing hormone agonist. This further documents the role of gonadal steroids in the growth of this tumor. (Am J Obstet Gynecol 1992;167:515-6.)

Key words: Leiomyomatosis peritonealis disseminata, gonadotropin-releasing hormone, tumor regression

Leiomyomatosis peritonealis disseminata is a rare condition in which multiple small nodules consisting of benign smooth muscle are found throughout the abdominopelvic viscera and peritoneum. Only two malignant cases have been reported.[1] This is the first report documenting decreased nodule growth with a gonadotropin-releasing hormone (GnRH) agonist.

Case report

A 29-year-old woman, gravida 1, para 0-0-1-0, presented with an 8-year history of intermittent and increasing left lower abdominal pain. The pain was colicky in nature and had no notable aggravating or alleviating factors. The patient did not have nausea, vomiting, or abnormal weight loss. She did report previous irregular bleeding and had been taking oral contraceptives since 1981. In 1983 she had a cervical fibroid removed by curettage. Pathologic studies confirmed a pedunculated leiomyoma. Because of persistent abdominal complaints, the patient underwent laparoscopy in 1989, which revealed disseminated peritoneal leiomyomatosis (Fig. 1). Multiple small smooth muscle tumors measuring 0.5 to 2 cm in diameter were noted on the ovaries, oviducts, anterior and posterior cul-de-sac, sigmoid colon, round ligaments, uterus, small intestines, and pelvic side walls. Biopsies showed the tumors to be benign.

Because of the known association between estrogen and progression of this disorder, the patient was placed on a regimen of leuprolide acetate (Lupron), a GnRH agonist, 0.5 ml subcutaneously daily to shrink tumor implants by decreasing estrogen production. Lupron

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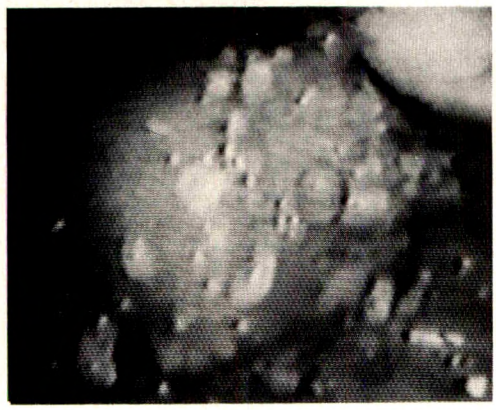


Fig. 1. Disseminated peritoneal leiomyomatosis in case 1.

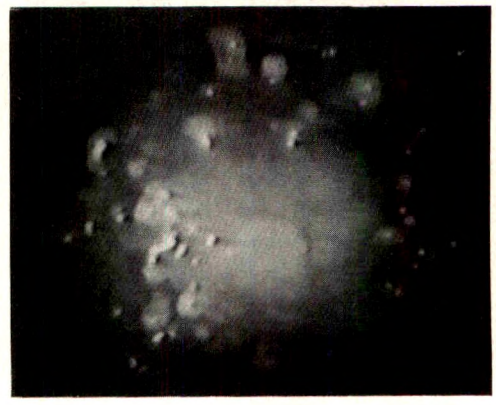


Fig. 2. Decreased leiomyomatosis after therapy in case 2.

therapy was administered for 6 months with notable hot flashes and persistent abdominal discomfort. A second laparoscopy was performed in June 1990. The lesions were significantly smaller in size; however, they were still present. Implants were no larger than 1 cm. Biopsies were performed and the tumors were found to be benign.

Other forms of ovarian suppression medroxyprogesterone acetate (Depo-Provera), megestrol acetate (Megace), and norethindrone (Micronor) were all tried over the next 6 months with notable side effects and continued discomfort.

In November 1990 total abdominal hysterectomy, bilateral salpingo-oophorectomy, and appendectomy were performed. At surgery a return to the pre-GnRH appearance was noted, with multiple 0.5 to 2 cm leiomyomas throughout the pelvis. Pathologic sections revealed virtually identical microscopic findings with discrete nodular proliferation of relatively hypocellular smooth muscle. No nuclear pleiomorphism was noted, and there was no evidence of significant mitotic activity. Two months after operation the patient was reinitiated on a regimen of low-dose estrogen therapy. In June 1991 a repeat laparoscopy was performed, revealing persistent leiomyomatosis in the pelvis, although greatly decreased from the time of hysterectomy, with leiomyomas measuring only 3 to 4 mm in diameter (Fig. 2). In September 1991 the patient returned for follow-up and reported occasional right lower-quadrant pain but normal bowel function. A pelvic examination showed significant improvement from prior examinations.

Comment

This case report further confirms the association of estrogen with the progression of disseminated leiomyomatosis peritonei. This condition is believed to be a hormonally stimulated disease process that is usually found in women who are either pregnant or using hormonal contraception. Oral contraception was discontinued and Lupron, a GnRH agonist, was administered to decrease gonadal steroid production. In this case regression of tumor volume was noted while the patient was receiving the GnRH agonist; however, no significant subjective improvement in the patient's symptoms was noted. This condition has been reported 43 times with only two of the incidents ($p = 0.05$) demonstrating malignant degeneration.[1] This case represents the first documented case of regression of tumor mass by a GnRH agonist.

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A case of hidradenitis suppurativa treated with gonadotropin-releasing hormone agonist and by total abdominal hysterectomy with bilateral salpingo-oophorectomy

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We report on a patient with hidradenitis suppurativa with premenstrual exacerbations, who was treated successfully with total abdominal hysterectomy and bilateral salpingo-oophorectomy combined with estrogen substitution. As a trial, she was initially treated successfully with a gonadotropin-releasing hormone agonist and subsequently a gonadotropin-releasing hormone agonist in combination with estrogen substitution. (AM J OBSTET GYNECOL 1992;167:517-8.)

Key words: Hidradenitis suppurativa, gonadotropin-releasing hormone, hysterectomy, salpingo-oophorectomy

Hidradenitis suppurativa is a chronic inflammatory disease of skin areas with apocrine sweat glands, especially the axilla and the anogenital region. It is characterized by deep abscesses, healing with scar and sinus tract formation. There is a female preponderance, and in women the axilla is the most involved area. In general, the disease has a postpubertal onset, and most of the patients with a regular menstrual cycle experience premenstrual flares. Treatment consists primarily of local and systemic antibacterial measures; however, additional surgery is often indicated. Sometimes even total excision of the apocrine sweat gland–bearing areas is warranted. However, this therapy fails in many cases. Treatment with isotretinoin, and hormonal treatment with cyproterone acetate is not entirely satisfactory. Treatment with oral contraceptives may benefit some patients; however, in some women the disease will develop while they are using the pill.[1] We present a case in which the patient, after a successful trial with the gonadotropin-releasing hormone (GnRH) agonist buserelin acetate, was finally treated by total abdominal hysterectomy and bilateral salpingo-oophorectomy in combination with estrogen replacement therapy.

Case report

A 30-year old woman, para 0, abortus 2, was referred to our clinic because of premenstrual exacerbations of hidradenitis suppurativa. She had hidradenitis suppurativa since age 16. Medical treatment with several local and systemic antibacterial drugs and isotretinoin were all without success. She underwent surgery, including full-thickness skin excision and skinning vulvectomy, without a satisfactory result 99 times. Hormonal therapy with oral contraceptives, cyproterone acetate, tamoxifen acetate, medroxyprogesteron acetate depot, and oral progestogens had been tried without success. To test the possible steroid dependency of the disease, we prescribed the GnRH agonist buserelin acetate in a dose of 0.21 mg three times daily. During 10 months of treatment the disease went into regression and disappeared completely. She experienced mild vasomotor symptoms and other signs of estrogen deprivation. To test the hypothesis that the hidradenitis suppurativa is steroid dependent but not estrogen dependent, we added estradiol valerate (2 mg daily) to the GnRH medication. The disease remained in remission during the 3 months of treatment. After this trial the buserelin medication was terminated, and we decided to treat the patient by a total abdominal hysterectomy and bilateral salpingo-oophorectomy combined with estrogen replacement therapy with estradiol valerate. The operation was uneventful. Six months after surgery the patient was without complaint.

Comment

To the best of our knowledge this is the first report of hidradenitis suppurativa treated by total abdominal hysterectomy and bilateral salpingo-oophorectomy. In one case a patient with hidradenitis suppurativa was reported as successfully treated with a combination of the GnRH agonist leuprolide acetate and dexamethasone.[2] The authors reported giving preference to leuprolide over oral contraceptives because their patient

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suffered from congenital heart disease and other systemic diseases. They did not mention the planned duration of the GnRH therapy, as will be important in view of osteoporosis, cardiovascular disease, and unknown sequels by long-term use of GnRH agonists. Oral contraceptives had been tried in our patient without success. In contrast with oral contraceptives, progestogens, or antiandrogens, GnRH agonists suppress the ovaries without inducing steroids. Therefore we used a GnRH agonist to temporarily treat the patient and to predict the possible effect of bilateral oophorectomy. A trial of GnRH in combination with estrogen predicted that, for this patient, hormonal replacement therapy after total abdominal hysterectomy and bilateral salpingo-oophorectomy was feasible. Further study is needed to find the place of GnRH or hysterectomy with bilateral salpingo-oophorectomy in patients with persistent hidradenitis suppurativa. Although not proved, our trials suggest that progesterone may be a major cause in the development of hidradenitis suppurativa. So, when available, antiprogesterones may play a role in the treatment of hidradenitis suppurativa.

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Autonomic nervous system regulation of baseline heart rate in the fetal lamb

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OBJECTIVE: We examined 29 chronically instrumented fetal lambs from 125 to 143 days' gestation to investigate the effects of fetal behavioral states and autonomic nervous system maturation on baseline fetal heart rate.
STUDY DESIGN: Behavioral states were defined from electrocorticographic analysis as low-voltage fast activity or high-voltage slow activity. Decrease and increase in baseline fetal heart rate subsequent to administration of propranolol and methylatropine represented β-sympathetic and parasympathetic activity.
RESULTS: Baseline fetal heart rate decreased with gestation in both states, with steeper regression in low-voltage fast activity ($p < 0.001$). Positive correlation was noted between gestational age and percent decrease baseline fetal heart rate in both states with steeper regression in high-voltage slow activity ($p < 0.001$), and between gestational age and percent increase baseline fetal heart rate with steeper regression in low-voltage fast activity ($p < 0.001$). Fetal heart rate β-sympathetic and parasympathetic tones increased with age in both states, with elevation of β-sympathetic tone in high-voltage slow activity and parasympathetic tone in low-voltage fast activity.
CONCLUSION: Sympathetic and parasympathetic systems influence baseline fetal heart rate in these behavioral states and with age. (Am J Obstet Gynecol 1992;167:519-23.)

Key words: Baseline fetal heart rate, β-sympathetic tone, parasympathetic tone, fetal lamb, behavioral state, gestational age

Baseline fetal heart rate (FHR) declines with gestational age in the human fetus[1,2] and fetal lamb.[3] Schifferli and Caldeyro-Barcia[4] attribute this decline to maturing vagal parasympathetic functional control. Others report that baseline FHR is consistently higher in the high-voltage slow activity behavioral state than in the low-voltage fast activity state.[5-7] Using α-sympathetic and β-sympathetic blockades, Zhu and Szeto[8] concluded that the changes in baseline FHR relative to behavioral states were determined uniquely by sympathetic activity.[8] The regulation mechanisms of these fetal cardiovascular changes, especially the interaction of fetal behavioral states and autonomic nervous maturation on baseline FHR, remains unidentified. Our study investigated these control mechanisms at different gestational ages and behavioral states.

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Material and methods

Surgical preparation. Twenty-nine chronically instrumented fetal lambs between 125 and 143 days' gestation from time-mated, cross-bred Columbia-Rambouillet ewes were used in this study. Sheep gestation averages 145 days. All animals were maintained in a facility approved by the American Association for the Accreditation of Laboratory Animal Care and in accordance with *Guide for the Care and Use of Laboratory Animals* and met the standards of care of the U.S. Department of Agriculture. Before implementation research protocols were approved by the Animal Research Committee at the University of California, Irvine.

After 24 hours of food and water withdrawal anesthesia was induced with xylazine (Rompun) 12 to 14 mg administered intramuscularly. Lidocaine 1% was used locally as needed. The uterus was exposed with a midline abdominal incision. The fetal head was delivered through a uterine myometrial incision and covered with a surgical glove filled with warm saline solution. Bilateral electrodes were sutured under the skin in the fetal chest wall for electrocardiography. Paired stainless steel electrodes (ethylcyanoacrilate, Loctite

Corp., Newington, Conn.) were placed and cemented bilaterally in the fetal parietal skull for electrocorticography. The instantaneous FHR was obtained from the R-R interval of the electrocardiogram. Polyvinyl catheters were inserted into the fetal carotid artery, jugular vein, and trachea. The procedures were performed carefully to avoid disturbing the fetal vagal nerve. An open-end catheter was retained in the amniotic cavity, and additional catheters were placed in the maternal femoral artery and vein. All electrodes and catheters were exteriorized to the flank of the ewe.

Experimental protocol. The animals were maintained in an air-conditioned room and given free access to food and water. For 3 to 4 days after surgery the mother and fetus received ampicillin (200 mg/kg/day intravenously) and gentamicin (6 mg/kg/day intravenously), and ampicillin (1 gm/day) was administered to the amniotic cavity. Maternal and fetal arterial pH, blood gases, and hematocrits were checked daily. For pH and gas determinations approximately 0.6 ml arterial blood was collected anaerobically and measured (Corning pH/Blood Gas Analyzer, Model 168 BGA, Ciba Corning Diagnostic Corp., Medfield, Mass.) at 37° C. Fetal well-being was determined by the following criteria: pH >7.30. Po_2 >16 mm Hg, hematocrit >30%. Fetal arterial blood pressure, FHR, electrocorticogram, tracheal pressure, intrauterine pressure, and maternal blood pressure were continuously monitored on an 8-channel rectilinear recorder (Beckman Model R612, Sensor Medics Corp., Anaheim, Calif.). Animals that demonstrated interpretable electrocorticogram were selected for this study. Fetal behavioral states were analyzed from the electrocorticogram for low-voltage fast activity and high-voltage slow activity. Experiments were performed after allowing ≥5 days of postoperative recovery. All experiments were carried out between 9 AM and 5 PM with the ewe standing or lying quietly.

In 10 fetal lambs between 125 and 143 days' gestation baseline FHR was assessed under normal physiologic conditions in the low-voltage fast activity and high-voltage slow activity behavioral states. Baseline FHR was measured after FHR was stable for at least 10 minutes in which uterine contractions were absent. A set of observations during adjacent low-voltage fast activity and high-voltage slow activity was obtained every day after fetal well-being was confirmed. The average duration of observation per fetus was 6.3 days and the average number of observations per animal was 12.6.

In 10 fetal lambs between 126 and 143 days' gestation saline solution was infused via the fetal jugular vein at 4.0 ml per hour for a 2-hour control period. The fetus then received an intravenous priming dose of propranolol (1.0 mg, 1 ml) followed by propranolol infusion (2.0 mg/hr, 4.0 ml/hr) for 2 hours to block β-sympathetic control of the heart rate. Baseline FHR was obtained in low-voltage fast activity and high-voltage slow activity states during control and propranolol periods. The percent decrease in baseline FHR between control and propranolol periods was calculated as an index of FHR β-sympathetic tone. Completeness of the β-blockade was indicated by the absence of cardiac acceleration response to an injected β-agonist (isoproterenol 2.0 μg/kg estimated fetal weight). The experiment was repeated on the same fetuses in 2 to 5 days, confirming complete recovery from the blockade. The average number of experiments per fetus was 4.5.

In nine fetal lambs between 127 and 143 days' gestation a 2-hour control period was followed by methylatropine (200 μg/kg estimated fetal weight intravenously) for blockage of the parasympathetic control of the heart rate. The methylatropine dose was determined in preliminary experiments. Baseline FHR was measured in the low-voltage fast activity and high-voltage slow activity states during the control period and subsequent to methylatropine administration. The percent increase of baseline FHR from control to methylatropine periods was taken as representing FHR parasympathetic tone. Completeness of methylatropine blockade was evidenced by the absence of deceleration response to norepinephrine (4 μg/kg estimated fetal weight). The effect of the blocking dose in our study lasted for ≥3 hours. The experiment was repeated on the same fetuses in 2 to 5 days, confirming complete recovery from the blockade. The average number of experiments per fetus was 4.5.

Data analysis. Regression lines were determined by the least squares method. The regression slopes were compared by t test regression analysis.[10] Statistical significance was accepted at $p < 0.05$.

Results

Figure 1 shows the normal physiologic relationship of fetal gestational age and baseline FHR in low-voltage fast activity (LVFA) and high-voltage slow activity (HVSA). Baseline FHR in both behavioral states decreased with gestational age. The regression equations for baseline FHR (BFHR) versus gestational age (GA) are as follows:

$$\text{LVFA:}\quad \text{BFHR} = -4.47(\text{GA}) + 745.97 \quad (r = 0.79,\ n = 126,\ p < 0.001)$$

$$\text{HVSA:}\quad \text{BFHR} = -2.44(\text{GA}) + 488.84 \quad (r = 0.47,\ n = 126,\ p < 0.001)$$

The low-voltage fast activity slope (−4.47) was significantly less ($p < 0.001$) than that of the high-voltage slow activity slope (−2.44).

Figure 2 shows the influence of gestational age on baseline FHR response to β-sympathetic blockade with propranolol infusion in the low-voltage fast activity and high-voltage slow activity states. A positive correlation was found between gestational age and percent de-

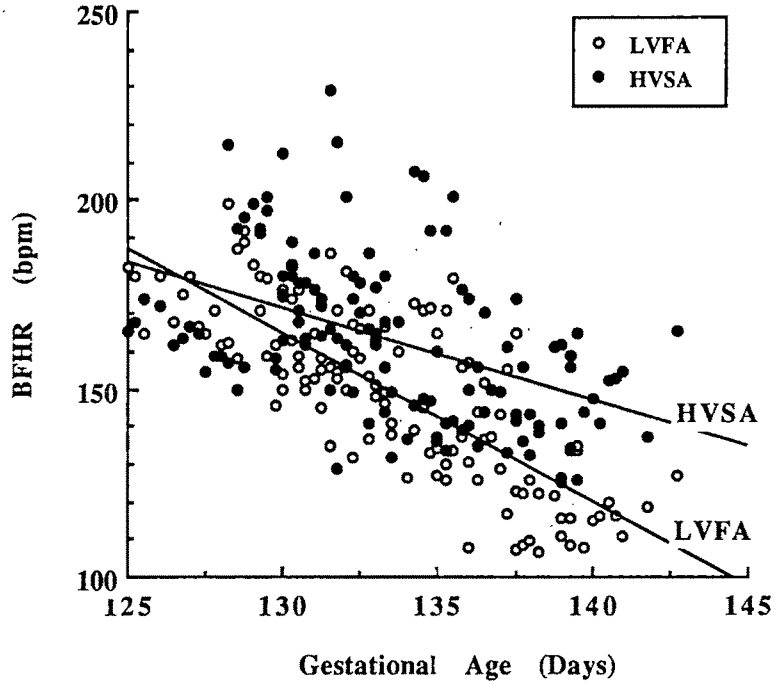


Fig. 1. Relationship between baseline FHR *(BFHR)* and gestational age in low-voltage fast activity *(LVFA)* and high-voltage slow activity *(HVSA)* states.

crease (% dec) in baseline FHR in both states. The regression equations follow:

LVFA: % dec BFHR = 0.54(GA) − 62.86 ($r = 0.50$, $n = 45$, $p < 0.001$)
HVSA: % dec BFHR = 0.97(GA) − 115.42 ($r = 0.65$, $n = 45$, $p < 0.001$)

The high-voltage slow activity slope (0.97) was significantly steeper than the low-voltage fast activity (0.54) ($p < 0.001$).

Figure 3 shows the relationship of gestational age and FHR response to parasympathetic blockade with methylatropine. The percent increase (% inc) in baseline FHR from control to methylatropine periods during low-voltage fast activity and high-voltage slow activity is illustrated. In both behavioral states the response increased with gestational age. The regression equations follow:

LVFA: % inc BFHR = 1.94(GA) − 243.28 ($r = 0.73$, $n = 30$, $p < 0.001$)
HVSA: % inc BFHR = 1.01(GA) − 129.59 ($r = 0.64$, $n = 30$, $p < 0.001$)

The low-voltage fast activity slope (1.94) was significantly steeper ($p < 0.001$) than the high-voltage slow activity (1.01).

Comment

Baseline FHR is regulated by the combined efforts of both intrinsic and extrinsic factors. The extrinsic factors are primarily the sympathetic and parasympathetic limbs of the autonomic nervous system.

The effects of the autonomic nervous system on baseline FHR in different behavioral states and gestational ages were assessed. Most previous studies used atropine sulfate to evaluate parasympathetic tone on FHR[11-13]; however, atropine sulfate has been reported to alter sleep behavior. Animals that received atropine sulfate did not show desynchronized sleep for several hours, and the desynchronized sleep that ultimately appeared was markedly modified.[14] For our study methylatropine was used as a parasympathetic blockade. This substance is much less lipid soluble and does not appear to act centrally.[15, 16] According to Guazzi et al.,[16] methylatropine (1 mg/kg) could be administered intravenously without any apparent sleep disturbance.[17]

In our study methylatropine 200 μg/kg estimated fetal weight was determined by prior experiments to be the appropriate dose. Complete parasympathetic block was obtained for ≥3 hours with no effect upon low-voltage fast activity– and high-voltage slow activity–cycling behavior. For the evaluation of autonomic nervous system effects on FHR, the sympathetic and parasympathetic blocking agents were not studied together. Twenty-four hours were allowed between tests for recovery of homeostasis. The percent decrease in baseline FHR subsequent to propranolol infusion and the percent increase in baseline FHR subsequent to methylatropine injection were calculated as representing FHR β-sympathetic and parasympathetic tone, re-

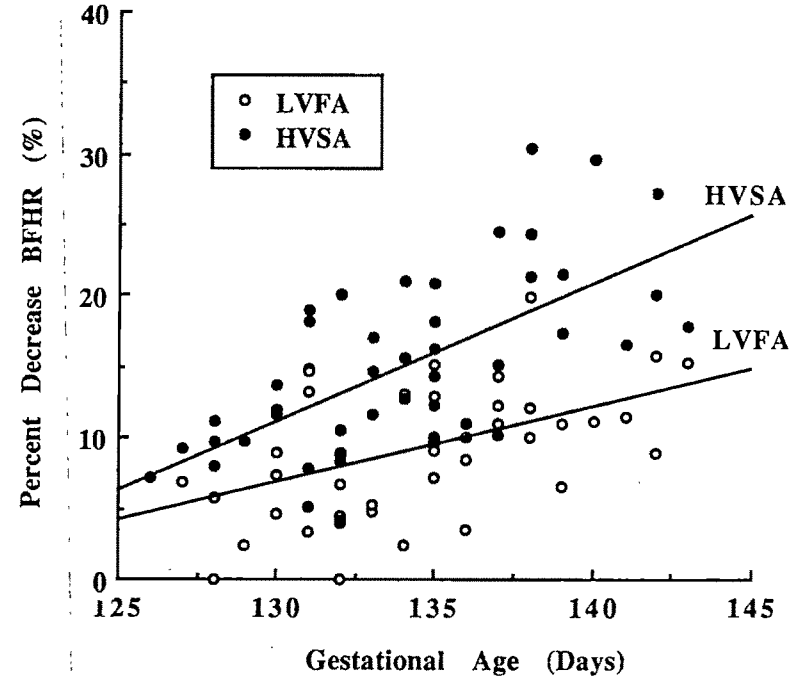


Fig. 2. Relationship between percent decrease in baseline FHR *(BFHR)* and in low-voltage fast activity *(LVFA)* and high-voltage slow activity *(HVSA)* states after propranolol.

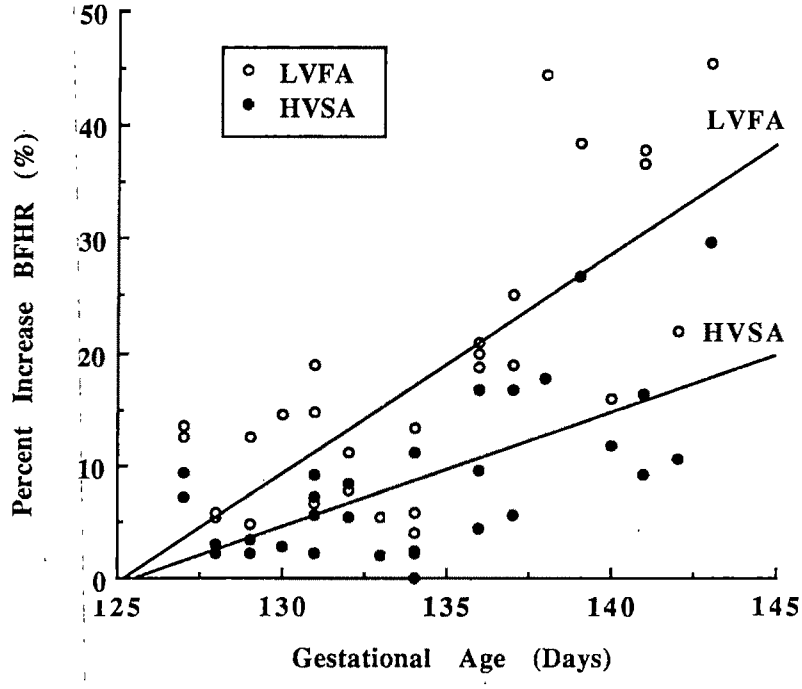


Fig. 3. Relationship between percent increase in baseline FHR *(BFHR)* and gestational age in low-voltage fast activity *(LVFA)* and high-voltage slow activity *(HVSA)* states after atropine.

spectively.[11-13] The percent decrease in baseline FHR increased with gestational age, and the slope of the regression line was steeper in the high-voltage slow activity state. These data indicate that FHR β-sympathetic tone increases with gestational age and is greater in the high-voltage slow activity state. The difference between low-voltage fast activity and high-voltage slow activity sympathetic tone also increased with gestational age.

The percent increase in baseline FHR rose with gestational age, and the regression line slope was steeper during the low-voltage fast activity state. These results

indicate that FHR parasympathetic tone increases with gestational age. FHR parasympathetic tone was higher in low-voltage fast activity, and the difference between states increased with gestational age.

A number of studies have reported conflicting results about the autonomic system influence on FHR. The existence of adrenergic cardiovascular function in the fetal lamb as early as 85 days' gestation was demonstrated by bradycardia response to propranolol.[13] Parasympathetic cardiac function in the fetal lamb at 60 days' gestation was shown by vagally driven heart rate slowing.[11] Nuwayhid et al.[12] reported that parasympathetic tone on resting heart rate in the fetal lamb was feeble at <130 days and sympathetic tone was constant throughout gestation. Walker et al.[3] found a progressive increase in parasympathetic tone and decrease in sympathetic tone toward term in the fetal lamb.[1] Vapaavouri et al.[13] reported that parasympathetic tone is fully developed by 120 days' gestation in the fetal lamb and that sympathetic tone increased from 101 days on. Other studies have reported that sympathetic influence upon FHR is significant[18] or minor.[19] In our study gestational age correlated positively with increased sympathetic and parasympathetic tone from 85% of gestational period to term. These results demonstrate that autonomic effects on FHR develop markedly during the last 15% of gestation in the fetal lamb. Assali et al.[11] concluded that sympathetic tone becomes active earlier than parasympathetic tone, and Walker et al.[3] showed a decrease of sympathetic influence toward term that resulted from a strong increase in parasympathetic activity. They did not, however, consider differences associated with behavioral states. In our study FHR sympathetic tone was more notable during high-voltage slow activity and parasympathetic tone more pronounced during low-voltage fast activity. Thus each behavioral state has unique autonomic effects upon FHR.

It would appear that FHR in the absence of autonomic effector action declines progressively with gestational age and that this may be a function of increased cardiac muscle mass and cardiac work. In our study we conclude that with behavioral state changes shifts in relative magnitudes of the simultaneous parasympathetic inhibitory and sympathetic acceleratory influences modulate the basal or intrinsic heart rate. During the low-voltage fast activity state increases in parasympathetic activity and decreases in sympathetic activity result in lower baseline FHR. On the other hand, during high-voltage slow activity state sympathetic activity increases and parasympathetic activity decreases so that baseline FHR rises.

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Interferon-γ receptors on human gestational choriocarcinoma cell lines: Quantitative and functional studies

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OBJECTIVES: This study was performed to further define the effects of interferon-γ on choriocarcinoma cell lines and to determine whether variations in response among cell lines are attributable to quantitative differences in interferon-γ receptors.

STUDY DESIGN: Interferon-γ receptors were quantified on BeWo, JEG-3 and Jar choriocarcinoma cell lines by a radiolabeled interferon-γ ligand binding assay. The response of these cell lines to interferon-γ was measured in two functional assays: a cell proliferation assay and a cell lysis assay after exposure to interferon-γ with and without actinomycin-D.

RESULTS: The number of interferon-γ receptors on BeWo, Jar, and JEG-3 cells did not differ significantly (650, 560, and 420 interferon-γ receptors per cell, respectively). Proliferation of all three choriocarcinoma cell lines was significantly inhibited to a similar extent by interferon-γ. After treatment with interferon-γ actinomycin-D, each choriocarcinoma cell line exhibited dose-dependent cell lysis; lysis of Jar was significantly less than that of either BeWo or JEG-3.

CONCLUSION: These data further document variations in the response of choriocarcinoma cell lines to interferon-γ and indicate that these differences are not the result of interferon-γ receptor number but of postreceptor mechanisms. (Am J Obstet Gynecol 1992;167:524-30.)

Key words: Interferon-γ, receptors, choriocarcinoma

Gestational choriocarcinoma is unique both clinically and biologically. Choriocarcinoma is remarkably curable with chemotherapy even in the presence of widespread metastases.[1, 2] Furthermore, because choriocarcinoma cells express genes of paternal origin, choriocarcinoma is at least a partial allograft. The dramatic curability of this tumor may be attributable in part to a host immunologic response. The presence of drug-resistant choriocarcinoma may be related to patient-partner histocompatibility.[3, 4] The prognosis of patients with choriocarcinoma has been correlated with the intensity of lymphocytic infiltration at the tumor-host interface.[5, 6]

Immunologically competent T lymphocytes secrete a variety of cytokines, including interferon-γ when antigenically stimulated. Interferon-γ has been previously demonstrated to enhance class I human leukocyte antigen expression in some choriocarcinoma cell lines and to inhibit choriocarcinoma cellular proliferation in vitro.[7-9] Interferon-γ may therefore be an important mediator of host immune defense against choriocarcinoma cells. Although interferon-γ enhanced expression of class I major histocompatibility complex in BeWo and JEG-3 choriocarcinoma cell lines, it did not in the Jar choriocarcinoma cell line.[7] Therefore choriocarcinoma cell lines may be heterogeneous in their response to interferon-γ.

Our study was undertaken to further document differences between the choriocarcinoma cell lines in their response to interferon-γ and to determine whether variations in responsiveness of choriocarcinoma cell lines are attributable to differences in interferon-γ receptor number or postreceptor mechanisms. Interferon-γ receptors were quantified on choriocarcinoma cell lines by radiolabeled ligand binding assay; physiologic responses to interferon-γ were determined by two assays: measurement of cell proliferation after exposure to interferon-γ and cell lysis after exposure to interferon-γ with and without the addition of actinomycin-D to block internal protein lytic defense mechanisms.[10]

Material and methods

Cell lines and growth conditions. WISH cells (a human amnion cell line) and choriocarcinoma cell lines (Jar, JEG-3, BeWo) were obtained (American Type Culture Collection, Rockville, Md.). NIH/3T3 cells (a mouse fibroblast cell line) were kindly provided by Dr.

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Timothy Bestor, Harvard Medical School. WISH cells were grown and maintained in tissue culture medium containing Eagle's minimal essential medium (GIBCO, Grand Island, N.Y.) supplemented with 15% heat-inactivated fetal calf serum, L-glutamine (2 mmol/L), penicillin G sodium (100 U/ml), streptomycin sulfate (100 μg/ml), and 0.25 μg/ml amphotericin B (minimal essential medium–tissue culture medium). Choriocarcinoma cells were maintained in tissue culture medium consisting of Roswell Park Memorial Institute (RPMI) 1640 medium (GIBCO) containing 10% fetal calf serum, L-glutamine, penicillin G, streptomycin, and amphotericin B in the above-mentioned concentrations (RPMI–tissue culture medium). NIH/3T3 cells were cultured in high glucose Dulbecco's modified Eagle medium with 10% fetal calf serum and antibiotics (Dulbecco's modified Eagle medium–tissue culture medium). Cells were maintained as exponentially growing monolayer cultures at 37° C in a humidified atmosphere of 5% CO_2 and 95% air. They were routinely split twice or three times a week and were free from contamination with *Mycoplasma* (Hoechst 33258 stain fluorescence negative).

Interferon γ. Recombinant human interferon-γ (specific activity: 2.5×10^7 half maximal U/mg protein) was obtained (Genzyme, Boston). The purity of this preparation was certified by the manufacturer to be 99%. It was reconstituted with RPMI 1640 medium (10^5 U/ml) and stored at −70° C. Interferon-γ was diluted in RPMI–tissue culture medium before its use in the cytolytic assay. Recombinant human interferon-γ (New England Nuclear, Boston)[11] was radioiodinated by the Bolton-Hunter technique to a specific activity of 70 μCi/μg.

Actinomycin-D. The stock solutions of actinomycin-D (Sigma Chemical Co., St. Louis), were prepared weekly in RPMI 1640 medium containing supplements and kept in the dark at 4° C.

IFN-γ cell binding experiments. Human gestational choriocarcinoma cells (Jar, JEG-3, BeWo), WISH cells (positive control cells), and mouse 3T3 cells (negative control cells) were seeded into 35 mm 6-well tissue culture plates (Corning, N.Y.) in 1 ml appropriate tissue culture medium ($0.7\text{-}1 \times 10^6$ cells/well) and allowed to grow 24 to 48 hours until the cultures reached confluence (approximately 1.5×10^6/well). For saturation-binding studies, monolayers were washed twice with appropriate cold tissue culture medium and then incubated for 4 hours at 4° C or for 1 hour at 37° C with increasing concentrations (0.0036 nmol/L to 0.118 nmol/L) of iodine 125–labeled interferon-γ in 1 ml tissue culture medium. After incubation the binding reaction was terminated by aspirating the tissue culture medium and washing the wells carefully six times with cold tissue culture medium. The cells were detached with trypsin and counted on a hemacytometer; their radioactivity was counted in a gamma counter (Pharmacia LKB Nuclear, Gaithersburg, Md.). Cell viability was assayed by the trypan blue exclusion test. The concentration of ligand necessary for receptor saturation for each cell line was determined. To assess binding specificity, competitive binding assays were performed by incubating the cells for 4 hours at 4° C with ^{125}I-interferon-γ (0.012 nmol/L in 1 ml tissue culture medium), together with various concentrations (0.012 nmol/L to 6.2 nmol/L) of cold interferon-γ. The cells were then washed with Roswell Park Memorial Institute, detached, and counted as above. Nonspecific binding, defined as counts remaining in the presence of 500-fold excess of unlabeled interferon-γ, was subtracted from all readings, and the remaining counts were defined as specific binding. Nonspecific binding was <10% of total binding. The affinity constant and the number of receptors were calculated from the binding curves by Scatchard analysis.[12]

The cytolytic assay. The cytolytic assay used in these experiments is a modification of the techniques previously described by Massad et al.[10] In these experiments 200 μl of choriocarcinoma cell suspension (5×10^4 cells/ml RPMI–tissue culture medium) were seeded in 96-well, flat-bottomed remove-a-cell plates (Dynatech Laboratories, Inc., Chantilly, Va.) that had been precoated with laminin (Sigma, St. Louis). Cells were allowed to adhere to culture wells for 18 to 20 hours. The tissue culture medium was aspirated, the wells were washed once in RPMI and then 100 μl of interferon-γ dilution (10 to 10,000 units in 1 ml of RPMI–tissue culture medium) were added in triplicate. Control triplicate wells were plated simultaneously and incubated in RPMI–tissue culture medium alone. After 16 hours of incubation at 37° C, in 5% carbon dioxide, the cells were labeled with 100 μl of RPMI–tissue culture medium containing 75 μCi/ml sodium chromate 51 (New England Nuclear) in each well. After an additional 8 to 10 hours of incubation in a humidified chamber at 37° C in 5% carbon dioxide, the supernatant was aspirated and each well was washed five times in RPMI. 100 μl of RPMI–tissue culture medium were then added to each well and another 100 μl of RPMI–tissue culture medium containing 2×10^{-6} mol/L actinomycin-D were added to the experimental wells. Control wells only received 100 μl of RPMI–tissue culture medium. After 24 hours of incubation at 37° C in a humidified atmosphere of 5% carbon dioxide and 95% air, the plates were centrifuged at 40*g* for 4 minutes, and a 100 μl aliquot of supernatant was removed from each well and counted in a gamma counter. Total incorporation was determined by counting the radioactivity of 2.5×10^4 cells cpm. Spontaneous ^{51}Cr release was measured in wells that were not pretreated with

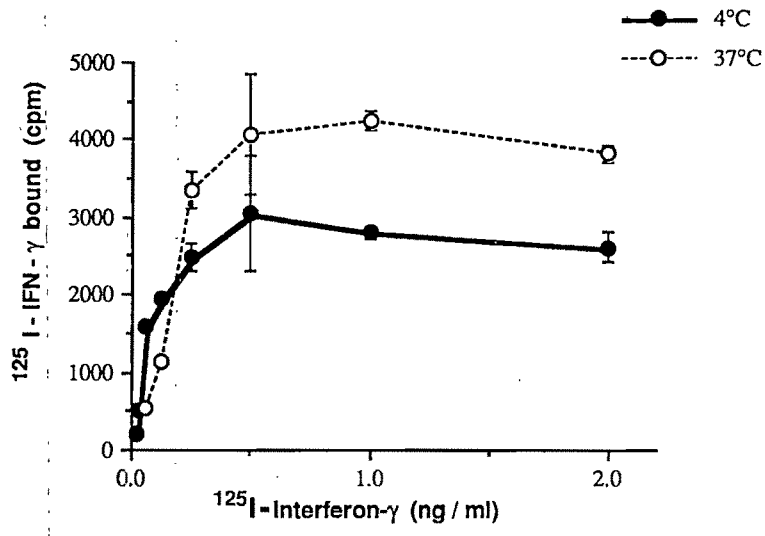


Fig. 1. Binding of ^{125}I-interferon-γ *(IFN-γ)* to Jar cells at 4° C and 37° C.

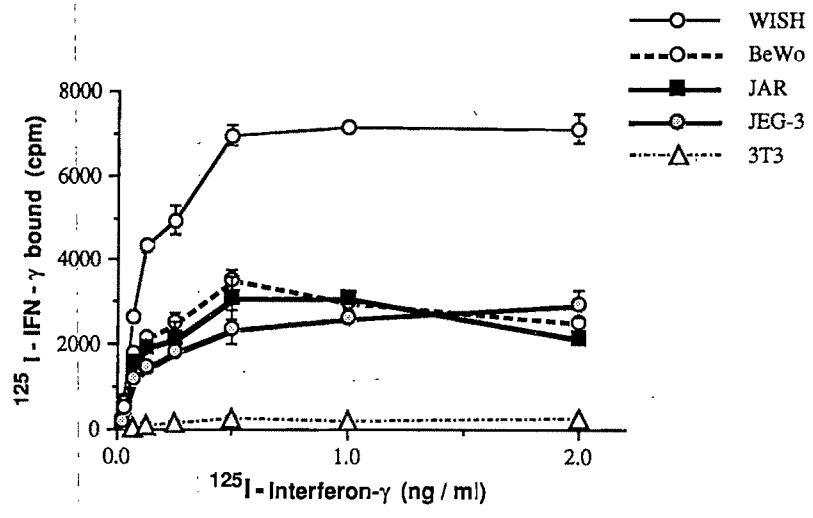


Fig. 2. Binding of ^{125}I-interferon-γ *(IFN-γ)* to WISH cells, gestational choriocarcinoma cells (Jar, JEG-3, BeWo), and mouse 3T3 cells at 4° C.

interferon-γ. Each assay was set up in triplicate, and the percentage of specific lysis (^{51}Cr release) was calculated as follows:

$$\text{Percent specific lysis} = \frac{\text{cpm } ^{51}\text{Cr (experimental)} - \text{cpm } ^{51}\text{Cr (spontaneous)}}{\text{cpm } ^{51}\text{Cr (total)} - \text{cpm } ^{51}\text{Cr (spontaneous)}}$$

The data were expressed as a mean percentage of specific lysis.

Tritiated thymidine incorporation assay. The tritiated thymidine incorporation assay with JAR and BeWo choriocarcinoma cell lines was performed as previously described from our laboratory.[8]

Statistical analysis. For radio-receptor and cytolytic assays, data were analyzed with Statview II statistical software on a Macintosh SE/30 computer. Differences among groups were determined by two-way analysis of variance followed by Fisher's protected least significant difference post-hoc testing.

Results

Characteristics of interferon-γ binding and binding-site competition. The binding of radiolabeled interferon-γ to Jar cells was dose-dependent and reached saturation by 4 hours at 4° C and by 1 hour at 37° C. The amount of cell-associated interferon-γ was greater ($p < 0.05$) at 37° C (Fig. 1), possibly because of internalization of ligand. To standardize the data, subsequent binding assays were performed at 4° C for 4 hours. The binding of ^{125}I-interferon-γ varied among the cell lines tested, with WISH cells binding the most interferon-γ ($p < 0.05$). The mouse 3T3 cells had no

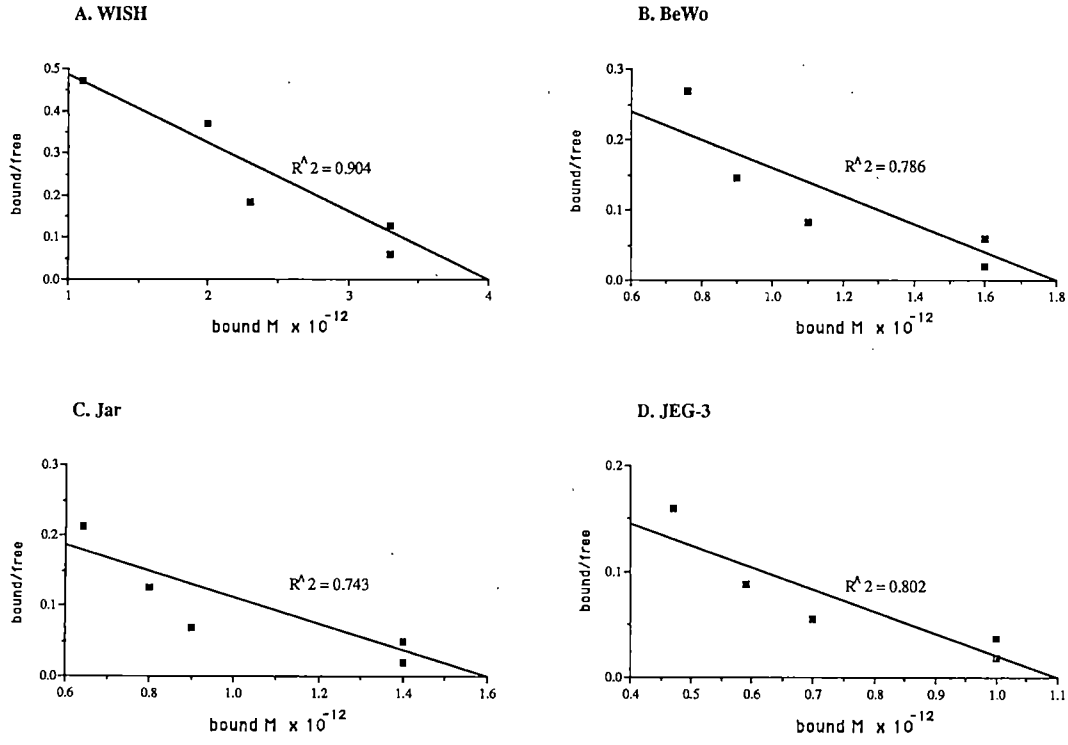


Fig. 3. Scatchard analysis of ^{125}I-interferon-γ binding to WISH and choriocarcinoma cell lines. *R^2*, Regression coefficient.

specific interferon-γ binding capacity, confirming the species specificity of this reaction (Fig. 2).

Scatchard analysis of the saturation curves showed a linear dependency (Fig. 3). These data indicate that there is a single type of high-affinity interferon-γ receptor on the human choriocarcinoma cell lines used in this study. Extrapolation toward the abscissa of the values at low interferon-γ concentrations gave a value of 1300 interferon-γ receptors per WISH cell with an affinity constant of 5.9×10^{-12} mol/L.[13] The same analysis revealed 650 interferon-γ receptors per BeWo cell, 560 interferon-γ receptors per Jar cell, and 420 interferon-γ receptors per JEG-3 cell, with affinity constants in the range of 3.3 to 4.9×10^{-12} mol/L. The differences in interferon-γ receptor numbers and affinity constants on the choriocarcinoma cell lines were not statistically significant ($p > 0.05$).

To establish the specificity of binding of ^{125}I-interferon-γ to choriocarcinoma cells a cold-competition assay was performed with the JEG-3 cell line. Unlabeled (cold) interferon-γ competitively inhibited the binding of ^{125}I-interferon-γ to JEG-3 cells in a dose-dependent manner, confirming binding specificity. The binding of ^{125}I-interferon-γ could be completely inhibited by a 50-fold excess of unlabeled interferon-γ (Fig. 4). The specific binding represented approximately 90% of the total counts bound.

Induction of lytic activity by interferon-γ on choriocarcinoma cell lines. The effects of various concentrations (10 to 10,000 U/ml) of interferon-γ alone, 10^{-6} mol/L actinomycin-D alone and the combination of interferon-γ and actinomycin-D were evaluated. The concentration of actinomycin-D was chosen based on prior reports describing the effect of actinomycin-D and interferon-γ on cervical carcinoma cell lines.[10] Spontaneous ^{51}Cr release was <14% for BeWo, <11% for Jar, and <6% for JEG-3 cells. No significant lysis was observed in response to interferon-γ alone at any concentration studied. The addition of actinomycin-D alone resulted in an increase in spontaneous chromium release with a low level of lysis. When interferon-γ was used in combination with actinomycin-D, marked enhancement of cell lysis was noted. This specific cell lysis was dependent on the concentration of interferon-γ used. Fig. 5 depicts a representative experiment where cells were pretreated for 24 hours with varying concentrations of interferon-γ. At interferon-γ ≥ 100 U/ml a dose-dependent increase in cell lysis was observed in the presence of 10^{-6} mol/L actinomycin-D in all three choriocarcinoma cell lines. However, the amount of cell lysis in the Jar cell line in the presence of actinomycin-D-interferon-γ was significantly less than that observed with the other choriocarcinoma cell lines ($p < 0.05$). Concentrations of interferon-γ <100 U/ml with 10^{-6} mol/L actinomycin-D caused the same low level of cell lysis as 10^{-6} mol/L actinomycin-D only.

Inhibition of proliferation of choriocarcinoma cell lines by interferon-γ. Proliferation in the Jar and

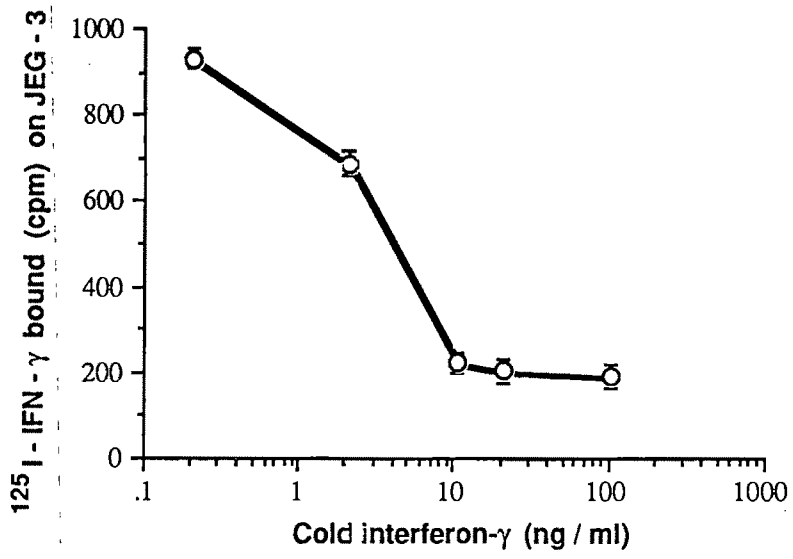


Fig. 4. Competitive inhibition of ^{125}I-interferon-γ *(IFN-γ)* (0.21 ng/ml) binding to JEG-3 choriocarcinoma cells by cold interferon-γ.

BeWo choriocarcinoma cell lines was significantly inhibited by interferon-γ (32% and 34% inhibition at 1000 U/ml, and 43% and 47% inhibition at 20,000 U/ml [$p < 0.05$]).

Comment

Interferon-γ is an important immunoregulatory cytokine produced by activated T cells and natural killer cells and plays an integral role in promoting specific mechanisms of host defense. Interferon-γ also regulates the production and activities of other immunomodulatory cytokines produced by macrophages and T cells such as tumor necrosis factor and certain interleukins.[14] In addition, interferon-γ produces antiproliferative effects in sensitive normal and neoplastic cells and is the major cytokine responsible for regulating expression of immunologically important cell surface determinants such as class I and II antigens of the major histocompatibility complex.[15]

We and others have previously demonstrated that class I human leukocyte antigen expression is up-regulated after exposure to interferon-γ in BeWo (the parent line of JEG-3[16]) and JEG-3 choriocarcinoma cell lines, but not Jar.[7, 9] This variation in response to interferon-γ may be attributable to differences in signal transduction pathways or other postreceptor mechanisms. To determine whether the variation in response to interferon-γ is unique to human leukocyte antigen expression, we used two different functional assays to further study the effects of interferon-γ in various choriocarcinoma cell lines. The interferon-γ cell lysis assay entailed the use of a protein synthesis inhibitor, actinomycin-D, which impairs a natural defense mechanism against cell lysis.[10] None of the choriocarcinoma cell lines demonstrated lysis in the presence of interferon-γ alone in concentrations ranging from 10 to 10,000 U/ml. In contrast, when incubated with actinomycin-D all three choriocarcinoma cell lines exhibited a dose-dependent increase in cell lysis with increasing concentrations of interferon-γ. However, the Jar cell line exhibited significantly less cell lysis with the actinomycin-D–interferon-γ combination than either BeWo or JEG-3 at concentrations of interferon-γ of ≥100 U/ml. The Jar cell line is therefore sensitive to lytic effects of interferon-γ but at a significantly lower level than the BeWo and JEG-3 cell lines. The tritiated thymidine incorporation studies performed in the current investigation and previously[8] indicated that proliferation is inhibited to a similar extent by interferon-γ in all three choriocarcinoma cell lines.

Interferon-γ induces its biologic effects by interacting with a specific interferon-γ receptor expressed at the target cell surface. The human interferon-γ receptor is an integral membrane glycoprotein with N-linked carbohydrates containing an extracellular ligand-binding domain with intramolecular disulfides necessary for ligand binding.[13] The amino acid sequence of the human interferon-γ receptor displays no resemblance to known proteins and does not belong to any of the known receptor families.[17] Unlike other inducible receptors for lymphokines, expression of interferon-γ receptors appears to be constitutive and is not influenced by agents inducing differentiation or by activation of cellular proliferation. Furthermore, receptor numbers do not substantially change during the cell cycle.[18] Under equilibrium conditions interferon-γ sensitive cells with high receptor numbers require proportionally lower interferon-γ concentrations to achieve sufficient

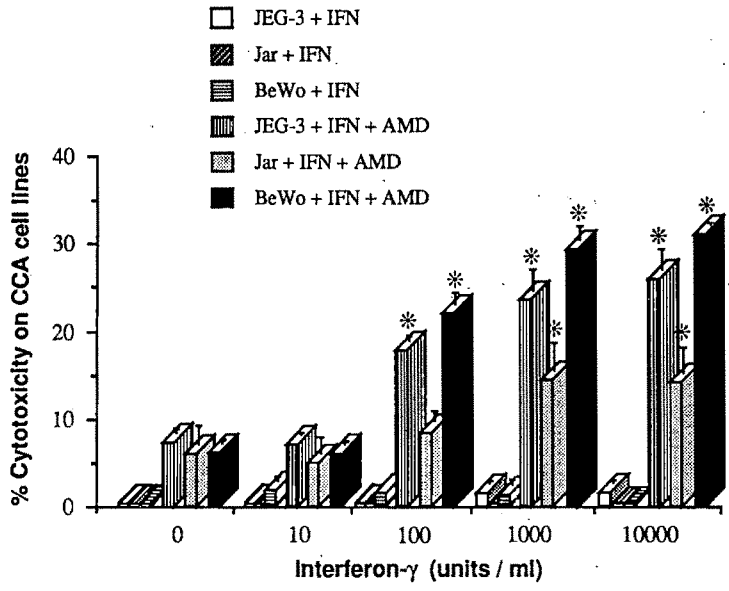


Fig. 5. Lytic effect of interferon-γ alone and in combination with actinomycin-D on three choriocarcinoma cell lines. *Asterisk,* Significantly greater percent cytotoxicity as compared with interferon-γ alone and actinomycin-D alone ($p < 0.05$).

receptor occupation-internalization for triggering a response as compared with cells with low receptor numbers. Together with the concentration of interferon-γ, the number of expressed receptors is critical for the induction of response. During the past few years a single class of high-affinity interferon-γ receptor has been demonstrated on a wide variety of cell types.[15] The mechanisms of postreceptor signal transduction by interferon-γ have not been clearly elucidated.

Human placenta is a rich source of interferon-γ receptor[19]; however, interferon-γ receptors have not previously been demonstrated on gestational choriocarcinoma cells. Using ^{125}I-interferon-γ with a high specific activity, we demonstrated that a receptor specific for human interferon-γ exists on gestational choriocarcinoma cells. The interferon-γ ligand bound with high affinity and in a highly specific and saturable manner to all three choriocarcinoma cell lines used in this study. The maximum binding capacity of ^{125}I-interferon-γ at 37° C was found to be nearly one and one half times more than measured at 4° C. The increased binding capacity at 37° C could be caused by de novo synthesis of additional interferon receptors; internalization of interferon receptors, or conformational changes of receptor or ligand molecules. There was no significant difference between the receptor numbers of BeWo, Jar, and JEG-3 lines; therefore the differing physiologic responses of choriocarcinoma cells to interferon-γ in human leukocyte antigen induction and lysis assays are not the result of quantitative differences in expression of the interferon-γ receptor.

The results of this study may have clinical relevance in the management of drug-resistant choriocarcinoma. Interferon-γ has antiproliferative and lytic effects on choriocarcinoma cells and also enhances their immunogenicity. Interferon-γ used alone or in conjunction wtih protein synthesis inhibitors such as actinomycin-D, a currently used agent in the treatment of choriocarcinoma, may play a role in the future therapy of this disease.

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Effects of sustained hypoxemia on the sheep fetus at midgestation: Endocrine, cardiovascular, and biophysical responses

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OBJECTIVE: The purpose of our study was to examine the effects of induced hypoxia on endocrine, cardiovascular, and biophysical measurements of the ovine fetus at 0.6 (83 to 93 days) of gestation anc to compare the fetal responses at this earlier gestation with those reported near term.

STUDY DESIGN: Fourteen fetal sheep were studied (9 in the hypoxia group and 5 in the control group) at 0.6 of gestation during a 24-hour control period, 8 hours of either sustained hypoxemia or room air, and a 40-hour recovery period.

RESULTS: Induced fetal hypoxemia resulted in a progressive lactic metabolic acidosis; however, all fetuses had recovered within 24 hours. The fetal endocrine response was variable with norepinephrine, the only measured hormone showing a significant hypoxia-related increase ($p < 0.05$). Fetal heart rate and mean arterial blood pressure showed little hypoxia-induced change, although fetal heart rate was significantly increased over the first 2 hours ($p < 0.05$). The percent time fetal breathing movements, electroocular activity, and nuchal muscle activity likewise showed little hypoxia-induced change.

CONCLUSION: The cardiovascular and biophysical response of the preterm fetus to induced hypoxemia is thus much less pronounced than that of the older gestational-aged fetus; this difference may impact on survival and the success of antenatal assessment protocols. (Am J Obstet Gynecol 1992;167:531-40.)

Key words: Fetal biophysical activity, breathing movements, heart rate

The response of the ovine fetus to induced hypoxemia is well characterized near term with a decrease in both breathing[1-5] and body movements[6] when fetal oxygenation is sufficiently compromised. This response results in a protective decrease in oxygen requirements[7,8] and provides a physiologic basis for the use of biophysical parameters in monitoring the high-risk pregnant patient. However, controlled animal data for the preterm fetus is lacking in spite of the improved perinatal survival and associated need for antenatal assessment at younger gestational ages for human pregnancy. Therefore the purpose of our study was to examine the effects of induced hypoxia on biophysical and cardiovascular measurements of the ovine fetus at 0.6 (83 to 93 days) of gestation and to compare fetal responses at this earlier gestation with those reported near term.

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Material and methods

Surgical procedures. Fourteen fetal lambs of mixed breed (nine in the hypoxia group; five in the control group) were surgically prepared for study between 78 and 87 days of gestation (term = 147 days). Ewes were given 600 mg of thiopental sodium intravenously; the ewes were intubated and maintained on a closed-circuit anesthesia system with 0.5 to 1.0% halothane (Abbott Laboratories, Montreal) and 67% oxygen–33% nitrous oxide at 6 L/min. Under sterile conditions a midline incision was made in the ewe's abdomen, and the uterus was exposed and opened. Polyvinyl catheters were placed into the fetal carotid artery (V3, Bolab, Lake Havasu City, Ariz.), trachea, jugular vein (V4, Bolab), and amniotic cavity (V11, Bolab). Electrodes of stainless steel polytetrafluoroethylene-coated wire (Cooner, Chatsworth, Calif.) were implanted through the orbital ridge of the zygomatic bone of each eye for recording of the electrooculogram, into the nuchal muscle for recording the nuchal muscle electromyogram, and over the sternum for recording the fetal heart rate. A reference electrode was placed in the loose connective tissue around the neck. The catheters (containing heparinized saline solution) and wires were exteriorized

through the flank of the ewe, and the abdomen was closed in layers. Polyvinyl catheters (V11, Bolab) were also placed in the maternal femoral artery and vein. At the time of surgery 800,000 IU of sodium penicillin G and 250 mg of streptomycin were injected intramuscularly to ewes and 1,000,000 IU of sodium penicillin G was injected into both the fetal jugular vein and the amniotic cavity. This antibiotic was administered daily until the experiment ended. All catheters were filled with sterile heparinized saline solution and flushed daily. Postoperatively, animals were maintained in individual cages suitable for continuous monitoring with food and water available. Ewes were also maintained on a 12-hour light-dark cycle and were allowed at least 4 days to recover from surgery before experiments began. Animal care followed the guidelines of the Canadian Council on Animal Care.

Physiologic measurements. All animals were studied during a 24-hour control period, an 8-hour experimental period of either sustained hypoxemia (hypoxia group) or normoxemia (control group), and a 40-hour recovery period. Continuous recording was obtained for fetal electroocular and nuchal muscle activities, tracheal pressure, carotid arterial blood pressure, amniotic fluid pressure, and fetal heart rate. Pressure transducers (Statham Model P-23 ID, Gould, Inc., Oxnard, Calif.) and a chart recorder (Model 7, Grass Instrument Co., Quincy, Mass.) were used to record pressures in the trachea, amniotic fluid, and carotid artery. Mean arterial blood pressure referenced to amniotic fluid pressure was calculated as diastolic plus 0.4 of systolic minus diastolic pressure. Electrical signals were processed with a common mode rejection preamplifier (Model 7, P51 IJ, Grass). Fetal heart rate was measured with a cardiotachometer (Model 7, 44B, Grass). Fetal electroocular and nuchal muscle potentials were displayed directly on the chart recorder after passage through a passive bandpass filter, 0.3 to 30 Hz, on the preamplifier.

After approximately 23 hours of normoxemic control period, a perspex chamber (volume 4.6 cu ft) designed to alter maternal-inspired gas concentration and to permit ewes to eat and drink was placed in the cage and perfused with air at 40 L/min as previously described.[9] For the hypoxia group, after 1 hour the gas mixture was changed to 9% oxygen, 3% carbon dioxide, and 88% nitrogen and maintained for 8 hours. Fifteen minutes before the initiation of induced hypoxemia, fetal and maternal arterial blood were sampled for blood gases, pH, hematocrit, oxygen saturation, glucose, lactate, corticotropin, cortisol, norepinephrine, and epinephrine (total 2.8 ml for the fetus, 5 ml for the mother). At 1 hour and 8 hours after the onset of induced hypoxemia, a second and third complete set of blood samples were drawn. Fetal arterial blood gases, pH, and oxygen saturation (0.3 ml) were also sampled every hour throughout the 8-hour period of induced hypoxemia to monitor fetal blood gas status. The perspex chamber was removed from the front of the cage. A fourth complete set of blood samples was taken 24 hours after the induced hypoxemia. In the control group similar procedures were followed except the ewes breathed room air in the perspex box at 40 L/min throughout the 8-hour experimental period. Biophysical monitoring was continued through 40 hours of recovery, with animals later killed and autopsies performed to examine for neuropathologic factors.

Maternal and fetal arterial blood samples were drawn into heparinized syringes and placed on ice. Arterial blood gases, pH, and oxygen and carbon dioxide concentration within the perspex plexiglass chamber were measured with a blood gas analyzer with temperature corrected to 39.5° C (ABL-3, Radiometer, Copenhagen). Oxygen content was calculated with a capacity of 1.36 ml oxygen/gm hemoglobin. Whole blood glucose and lactate measurements were made in triplicate with membrane-bound glucose oxidase and D-lactate dehydrogenase, respectively (model 23A, Yellow Springs Instrument Co., Yellow Springs, Ohio). Hematocrit was measured on a centrifuge (Autocrit Ultra 3, Clay Adams, Becton Dickinson, Parsippany, N.J.).

Blood samples for the determination of catecholamine concentrations were transferred to plastic tubes containing glutathione—ethylenediaminetetraacetic acid and centrifuged at 4° C for 15 minutes at 2000 rpm. The plasma was removed and stored at −70° C until analyzed. Blood samples for the determination of corticotropin and cortisol were transferred to plastic tubes without preservative and were similarly centrifuged and stored. Norepinephrine and epinephrine were subsequently measured by electrochemical detection after high-pressure liquid chromatography fractionation.[10] Immunoreactive corticotropin and cortisol were determined with radioimmunoassays as previously described and validated.[11]

Data analysis. Electroocular and nuchal muscle activities were assessed by visual analysis. Episodes of negative tracheal pressure were used to indicate fetal breathing movements. The minimum amplitude of breaths recognized was 1 mm Hg. Apnea was defined as an interval of >6 seconds with no episodes of fetal breathing movements. The incidence of fetal breathing movements, nuchal muscle, and electroocular activity were reported as the percentage of time spent in each 2-hour period. Fetal heart rate and arterial blood pressure recordings were analyzed every 5 minutes and reported as mean values for 1-hour intervals. Results are reported as the mean ± SEM. Data were analyzed by analysis of variance with repeated measures (BMDP 5V, BMDP Statistical Software Inc., Los Angeles).

Table I. Changes (mean ± SEM) in maternal arterial blood gases before, during, and after 8-hour period of experiments

	Before	*During*		*After*
	−0.25 hr	*1 hr*	*8 hr*	*24 hr*
Control (n = 5)				
pH	7.45 ± 0.02	7.45 ± 0.01	7.45 ± 0.01	7.46 ± 0.02
P_{CO_2} (mm Hg)	33.8 ± 1.6	35.4 ± 0.9	34.6 ± 1.3	33.4 ± 0.8
P_{O_2} (mm Hg)	113 ± 3	116 ± 3	124 ± 2	118 ± 4
Base excess (mmol/L)	0.2 ± 1.3	1.1 ± 1.0	2.4 ± 0.4	−0.1 ± 0.5
Hypoxia (n = 9)				
pH	7.43 ± 0.02	7.50 ± 0.01*	7.51 ± 0.01*	7.46 ± 0.01
P_{CO_2} (mm Hg)	35.1 ± 2.1	30.1 ± 1.6*	27.8 ± 1.3†	34.7 ± 1.6
P_{O_2} (mm Hg)	122 ± 3	61 ± 6†	46 ± 3†	113 ± 3
Base excess (mmol/L)	0.6 ± 0.6	−0.6 ± 2.2	−0.1 ± 0.9	0.2 ± 0.9

*$p < 0.05$.
†$p < 0.01$.

When a significant F value ($p < 0.05$) was obtained mean values were compared by paired or unpaired t test with Bonferroni's correction. Some of the hormonal samples could not be processed because of technical difficulty. Data on the neuropathologic findings will be reported separately.

Results

Maternal blood gases and pH. The oxygen concentration in the perspex chamber was decreased from 21.0% ± 0.3% during the normoxemic control period to 9.7% ± 0.5% when measured 1 hour after the initiation of the induced hypoxemia. At the end of the 8-hour hypoxia period the oxygen concentration measured 8.7% ± 0.5%. The animals, although hyperventilating, appeared well and continued to feed and take water. For the control group the oxygen concentration in the perspex chamber was 22.5% ± 0.4% during the control period with little measured change thereafter. The maternal P_{O_2} fell significantly in the hypoxia group from 122 ± 3 to 61 ± 6 mm Hg after 1 hour, with a further fall to 46 ± 3 mm Hg by the end of the sustained hypoxemia (Table I). In spite of the carbon dioxide added to the perspex chamber, a relative respiratory alkalosis was evident through the 8 hours of induced hypoxia. In the control group there was little change in the maternal blood gases and pH throughout the period of study.

Fetal blood gases, pH, and oxygen concentrations. The mean gestational age at the time of study was 90.8 days for the control group and 89.6 days for the hypoxia group. The mean fetal weight at the time of animal death measured 980 gm and 932 gm, respectively, for the two groups. All fetuses survived through 40 hours of recovery. Two-way analysis of variance indicated a significant effect of both time and induced hypoxemia on fetal blood gases, pH, and oxygen concentrations (Table II, Fig. 1). Fetal arterial P_{O_2} decreased from 26.6 ± 1.1 mm Hg during the normoxemic control period to 17.3 ± 1.3 mm Hg at 1 hour, with a further decrease at 8 hours to 14.8 ± 1.0 mm Hg. There was a small but significant fall in fetal arterial P_{CO_2} likely caused by maternal hyperventilation because maternal arterial P_{CO_2} decreased a similar amount. Fetal arterial pH fell from 7.34 ± 0.01 during the normoxemic control period to 7.23 ± 0.03 8 hours after the induced hypoxemia; this fall can be attributed entirely to a developing metabolic acidosis, as indicated by the associated change in base excess and arterial lactate values. Fetal arterial oxygen content and oxygen saturation decreased in a manner similar to that of arterial P_{O_2} and hemoglobin values were little changed. All these measurements returned to control levels 24 hours after the induced hypoxemia. In the control group there was no significant change in any of these arterial blood measurements.

Fetal glucose and lactate concentrations. Two-way analysis of variance demonstrated a significant effect of both time and hypoxemia on lactate concentrations, but not on glucose concentrations (Table II). Fetal lactate concentration increased from 1.1 ± 0.1 mmol/L during the normoxemic control period to 9.6 ± 1.4 mmol/L 8 hours after the onset of induced hypoxemia, then decreased to 1.4 ± 0.2 mmol/L 24 hours after the sustained hypoxemia. In the control group fetal arterial glucose and lactate measured 1.1 ± 0.1 and 1.1 ± 0.2 mmol/L, respectively, with no change during the study period.

Fetal hormonal measurements. Mean fetal plasma corticotropin showed a variable response to the 8 hours of induced hypoxia, increasing fourfold or greater in four of the animals in which measurements were obtained, but with little change in the other three animals (Table III). Plasma cortisol values likewise were variably changed, increasing threefold or greater in three of the four animals in which corticotropin also increased, but

Table II. Changes (mean ± SEM) in fetal arterial blood gases, pH, oxygen concentration, glucose, and lactate before, during, and after 8-hour period of experiments

	Before	*During*		*After*
	−0.25 hr	*1 hr*	*8 hr*	*24 hr*
Control (n = 5)				
pH	7.36 ± 0.01	7.35 ± 0.01	7.36 ± 0.01	7.36 ± 0.02
P_{CO_2} (mm Hg)	43.5 ± 1.7	48.9 ± 1.2	49.8 ± 1.3	45.3 ± 0.9
P_{O_2} (mm Hg)	29.9 ± 2.1	31.9 ± 1.0	29.0 ± 0.9	29.7 ± 0.8
Base excess (mmol/L)	−0.4 ± 1.2	0.9 ± 0.9	2.4 ± 0.5	0.3 ± 0.8
Oxygen saturation (%)	73.3 ± 3.8	75.1 ± 3.5	65.4 ± 5.0	68.2 ± 3.7
Hemoglobin (gm/dl)	7.6 ± 0.4	7.9 ± 0.4	7.3 ± 0.5	7.5 ± 0.4
Oxygen content (mmol/L)	3.4 ± 0.2	3.6 ± 0.2	2.9 ± 0.3	3.1 ± 0.2
Glucose (mmol/L)	1.1 ± 0.1	1.2 ± 0.1	1.3 ± 0.1	1.3 ± 0.1
Lactate (mmol/L)	1.1 ± 0.2	1.0 ± 0.1	1.0 ± 0.2	1.1 ± 0.2
Hypoxia (n = 9)				
pH	7.34 ± 0.01	7.35 ± 0.01	7.23 ± 0.03†	7.33 ± 0.01
P_{CO_2} (mm Hg)	47.7 ± 1.0	42.4 ± 1.7*	39.1 ± 1.2†	47.7 ± 1.9
P_{O_2} (mm Hg)	26.6 ± 1.1	17.3 ± 1.3†	14.8 ± 1.0†	26.5 ± 1.7
Base excess (mmol/L)	−0.6 ± 0.5	−2.1 ± 0.6	−10.2 ± 0.9†	−0.9 ± 0.5
Oxygen saturation (%)	70.3 ± 2.4	44.3 ± 4.0†	25.9 ± 4.9†	65.3 ± 2.7
Hemoglobin (gm/dl)	8.0 ± 0.6	7.9 ± 0.6	8.3 ± 0.7	8.3 ± 1.1
Oxygen content (mmol/L)	3.4 ± 0.3	2.1 ± 0.3†	1.3 ± 0.2†	3.2 ± 0.4
Glucose (mmol/L)	1.0 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	1.1 ± 0.1
Lactate (mmol/L)	1.1 ± 0.1	1.5 ± 0.3	9.6 ± 1.4†	1.4 ± 0.2

*$p < 0.05$.
†$p < 0.01$.

with little change in the remaining animals. None of these changes were statistically significant on two-way analysis of variance with that of the control group. Maternal plasma corticotropin and cortisol showed no significant change, averaging 44 ± 5 pg/ml and 5.3 ± 0.4 ng/ml, respectively, for the hypoxia group animals and 42 ± 2 pg/ml and 5.3 ± 0.6 ng/ml, respectively, for the control group animals throughout the period of study.

Mean fetal plasma norepinephrine was significantly increased by 8 hours of induced hypoxemia, with a subsequent return to control values when measured 24 hours after the sustained hypoxemia ($p < 0.05$) (Table III). Although fetal plasma epinephrine values increased in each of the animals over the 8 hours of sustained hypoxemia, this did not reach significance because of the limited number of samples and variable increase noted. Maternal plasma norepinephrine and epinephrine both showed a variable response but without significant change, averaging 386 ± 111 pg/ml and 69 ± 14 pg/ml, respectively, throughout the period of study.

Fetal cardiovascular and biophysical activity measurements. In the hypoxia group mean fetal heart rate increased significantly from 196 ± 3 beats/min during the last hour of the normoxemic control period to 217 ± 6 beats/min over the first 2 hours of induced hypoxia, ($p < 0.05$) then returned to 208 ± 7 beats/min by 8 hours of sustained hypoxia (Fig. 2). Fetal mean arterial blood pressure was 34 ± 2 mm Hg during the last hour of the normoxemic control period and did not change significantly during the period of induced hypoxia. In the control group mean fetal heart rate and blood pressure were 199 ± 3 beats/min and 33 ± 1 mm Hg, respectively, during the last hour of the control period and showed no significant change throughout the period of study.

Satisfactory measurement of breathing movements, nuchal muscle activity, and electroocular activity were obtained from 12 studies (7 for the hypoxia group, 5 for the control group). Fig. 3 demonstrates a typical chart recording showing that fetal biophysical activity changed little during the 8 hours of induced hypoxemia. The incidence of fetal breathing movements was 20.5% ± 0.6% during the 72 hours of total recording for the control group; this incidence was similar to that during the 24 hours of normoxemic control for the hypoxia group (23.7% ± 1.4%) (Fig. 4). Two-way analysis of variance indicated no effect of induced hypoxia on fetal breathing movements, although a small drop was noted during the first 8 hours of recovery; 11.6% ± 3.3% and 18.7% ± 1.8% for the hypoxic and control groups, respectively. The incidence of nuchal muscle activity was 69.9% ± 1.4% of the time during the 72 hours of total recording in the control group, which was not different from the incidence of nuchal muscle activity during the 24 hours of normoxemic control for the hypoxia group (65.0% ± 2.1%) (Fig. 4). The incidence of electroocular activity was 81.9% ± 1.0% of the time during the 72 hours of total recording

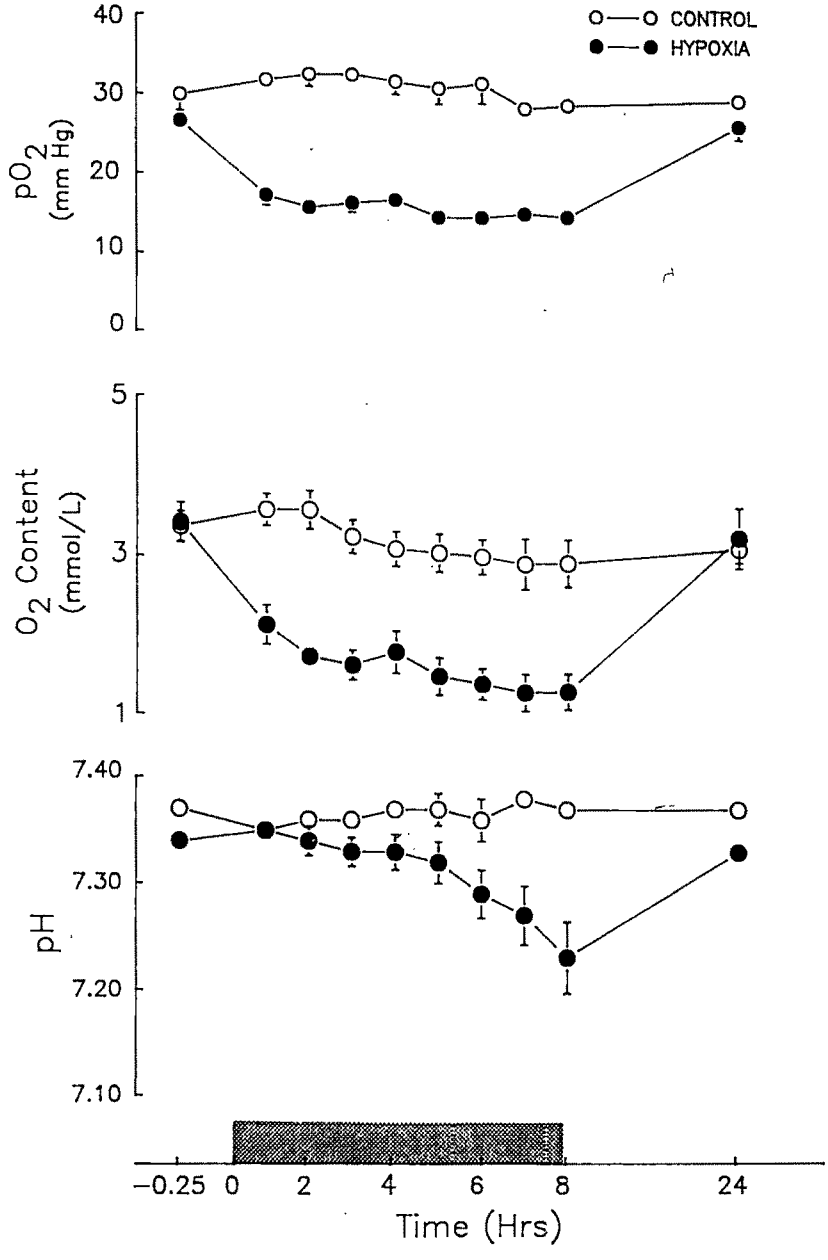


Fig. 1. Fetal carotid arterial Po_2, oxygen content, and pH (mean ± SEM) before, during, and after experiment *(shaded area)*. Induced hypoxemia resulted in significant reduction in fetal arterial Po_2, oxygen content, and pH.

in the control group, which again was not different from the incidence of electroocular activity during the 24 hours of normoxemic control for the hypoxia group (80.9% ± 1.2%) (Fig. 4). Two-way analysis of variance showed no significant effect of the induced hypoxia on either nuchal muscle or electroocular activities.

Comment

The use of the perspex chamber to lower maternal oxygen concentration allowed the animals continued access to food and water with little change in maternal corticotropin and cortisol as previously reported for animals similarly studied near term,[11] thus supporting this technique as a useful means of studying sustained fetal hypoxemia. In our study this was again induced by lowering the oxygen concentration within the chamber to 11% to 8%, with variation within this range to produce a developing metabolic acidosis as monitored by repeated sampling from the fetal carotid artery. The resultant maternal-inspired oxygen concentrations measured at 1 and 8 hours of hypoxia are similar to those we have reported for animals studied near

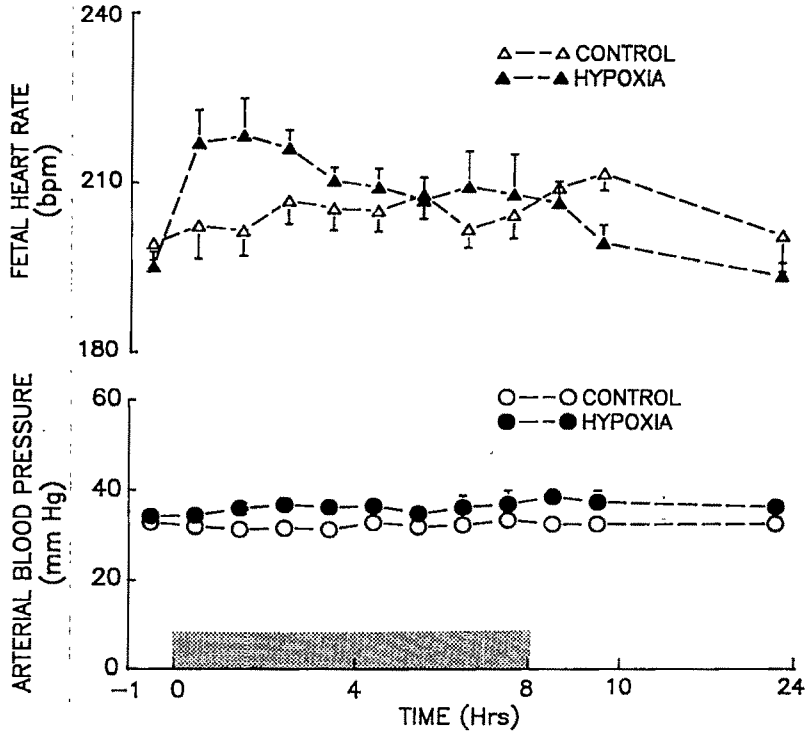


Fig. 2. Fetal heart rate and fetal carotid arterial blood pressure (mean ± SEM) before, during, and after experiment *(shaded area)*. Fetal heart rate and arterial blood pressure showed little hypoxia-induced change, although fetal heart rate increased significantly over first 2 hours of study.

Table III. Changes (mean ± SEM) in fetal plasma corticotropin, cortisol, norepinephrine, and epinephrine before, during, and after 8-hour period of experiments

	Before	*During*		*After*
	−0.25 hr	*1 hr*	*8 hr*	*24 hr*
Control (n = 4)				
Corticotropin (pg/ml)	24 ± 7	23 ± 8	42 ± 11	18 ± 5
Cortisol (ng/ml)	1.3 ± 0.3	1.3 ± 0.5	2.3 ± 0.8	2.1 ± 1.4
Hypoxia (n = 7)				
Corticotropin (pg/ml)	32 ± 7	40 ± 6	119 ± 36	54 ± 14
Cortisol (ng/ml)	1.6 ± 0.5	1.3 ± 0.2	2.0 ± 0.4	1.4 ± 0.2
Hypoxia (n = 6)				
Norepinephrine (pg/ml)	228 ± 37	369 ± 104	903 ± 276*	411 ± 103
Epinephrine (pg/ml)	21 ± 5	36 ± 14	88 ± 37	45 ± 18

Control values were not obtained for norepinephrine and epinephrine because of technical difficulties.
*$p < 0.05$.

term, as are the maternal blood gas and pH changes.[9]

Fetal arterial Po_2 and oxygen saturation values here measured at 0.6 of gestation under normoxemic control conditions are higher than in late gestation, a finding also noted in other studies of preterm animals[2, 12]; these higher values may relate in part to the relatively high umbilical blood flow in relation to fetal weight at this gestational age.[12] Conversely, fetal hemoglobin concentration tends to be lower in the preterm fetus[12] as also noted in our study. Fetal arterial Po_2 decreased acutely and measured 17.3 mm Hg when sampled at 1 hour of induced hypoxemia, with a further fall to 14.8 mm Hg when measured at 8 hours. Arterial pH fell after the first hour; this fall can be attributed to a developing metabolic acidosis as indicated by the associated change in base excess and arterial lactate values. Fetal arterial oxygen content also fell throughout the period of induced hypoxia, reflecting the drop in arterial Po_2 and the presumed rightward shift in the oxyhemoglobin dissociation curve with the developing metabolic acidosis. Although the imposed decrease in

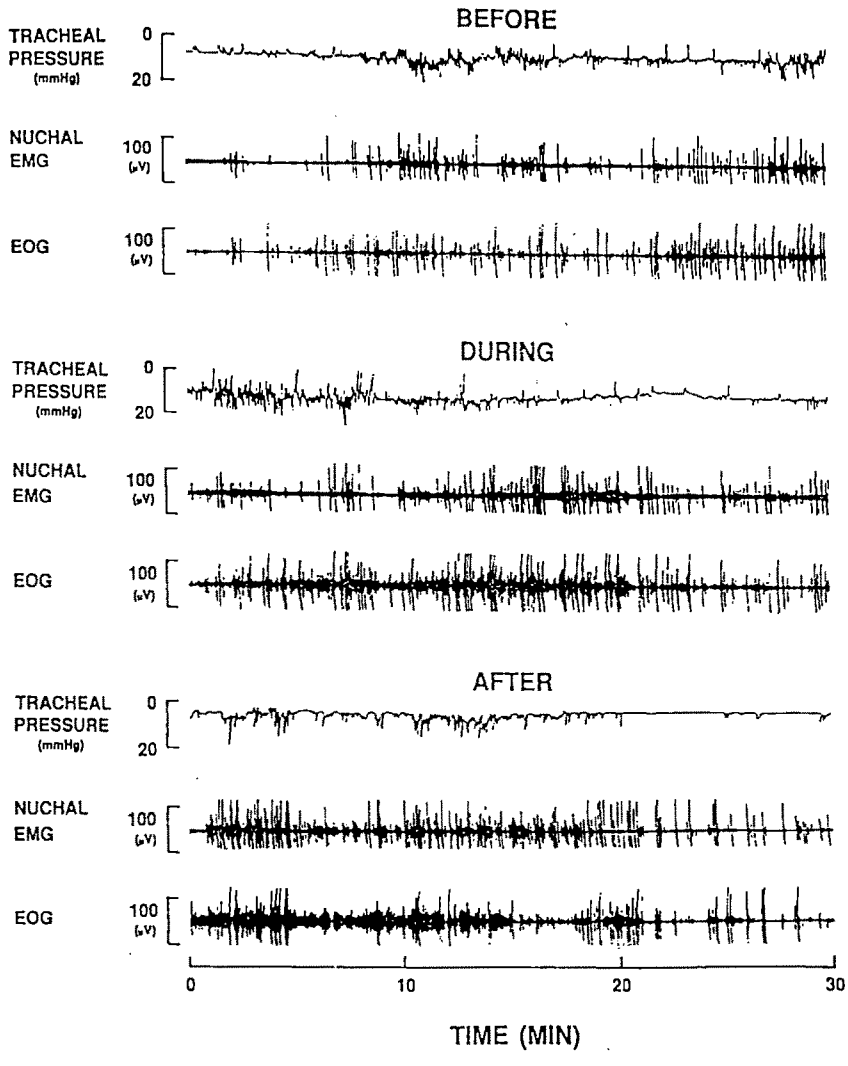


Fig. 3. Tracheal pressure, nuchal muscle electromyogram *(Nuchal EMG)*, and electrooculogram *(EOG)* 2 hours before *(BEFORE)*, 7 hours after *(DURING)*, and 24 hours after *(AFTER)* the onset of induced hypoxia in a sheep fetus at 87 days of gestation.

maternal-inspired oxygen was similar to that of animals we have previously studied near term,[9] neither the fall in fetal oxygenation nor the degree of acidemia was as pronounced. This finding of a developmental change in the fetal blood gas response to a given hypoxic insult was also noted by Clewlow et al.[2]; again, it may relate to the relatively high umbilical blood flow of the preterm fetus and thus an increased oxygen margin of safety. Fetal arterial glucose levels, which increase progressively in response to induced hypoxemia near term,[9] showed little change in our study. This lack of change may relate to the less pronounced fall in fetal oxygenation and thus degree of acidemia or an immaturity of hormonal responses to hypoxia at midgestation, including that of catecholamines as noted in our study and which are known to impact on fetal glucose levels.[13]

Fetal plasma immunoreactive corticotropin concentrations here measured under resting conditions are somewhat higher than we have reported in later gestation.[11] Although our antibody is specific and recognizes corticotropin within the region,[18-24] there may be cross-reactivity with circulating larger molecular-weight species of corticotropin at earlier gestational ages. The variable response of corticotropin to hypoxemia and the overall diminished corticotropin response compared with older fetuses[11] is consistent with reduced pituitary responsiveness to corticotropin-releasing hormone in younger fetuses.[14] At 90 days' gestation the fetal adrenal gland likewise shows decreased responsiveness to corticotropin; it is likely that much of the circulating cortisol in the fetus at this time is derived by transplacental transfer from the mother.[14]

Fetal plasma catecholamine levels here measured under resting conditions are somewhat lower than those reported by Iwamoto et al.[15] in preterm animals at 90

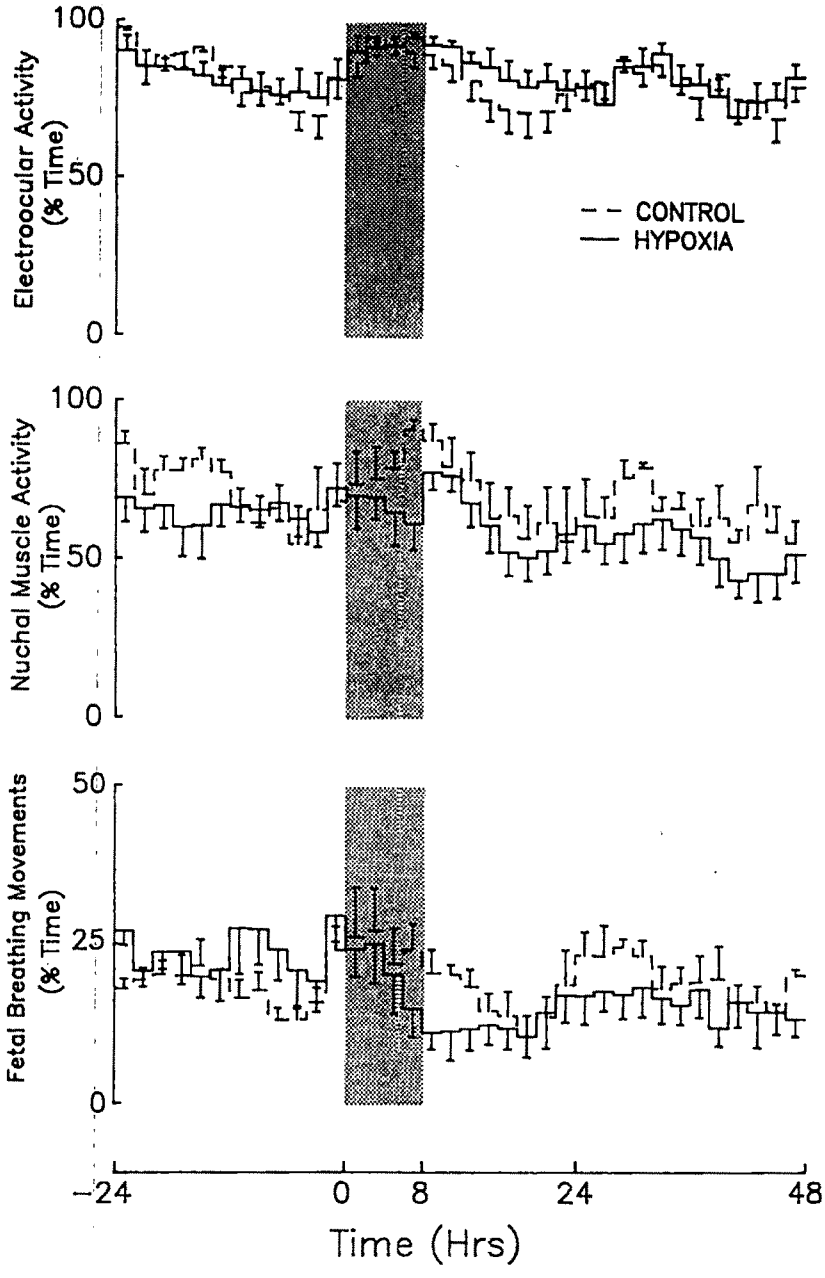


Fig. 4. Incidence of electroocular, nuchal muscle activities, and fetal breathing movements (mean ± SEM) per 2-hour interval plotted continuously from 24 hours before to 48 hours after onset of experiment *(shaded area)*. All variables showed little hypoxia-induced change.

days' gestation, although the levels are similar to those reported by Lewis et al.[16] in slightly older gestational-aged animals. In response to hypoxemia of 8 hours' duration plasma catecholamine levels were increased approximately fourfold, which is greater than the twofold increase noted by Iwamoto et al.,[15] possibly reflecting the different durations of induced hypoxemia, 8 hours versus approximately 1 hour, and the degree of associated metabolic acidemia, arterial pH 7.23 versus 7.32. This hypoxia-related increase in fetal catecholamine levels at 0.6 of gestation is nonetheless of lower magnitude, both from the standpoint of change in absolute levels and percent change from resting levels than in animals similarly studied near term[16] in keeping with an age-dependency as recently reported by Widmark et al.[17]

The mean fetal heart rate reported here is higher, whereas mean arterial blood pressure is lower than in late gestation, findings noted in other studies of preterm animals and attributed to a lower vagal tone and systemic vascular resistance, respectively.[1, 12, 18] In fetuses older than 120 days of gestational age, acute hy-

poxemia increases cholinergic and adrenergic influence on fetal heart rate, but the increase in cholinergic activity initially predominates and heart rate decreases.[19] It has been suggested by Walker et al.[19] that between 109 and 120 days of gestation the influence of cholinergic mechanisms is less than the influence later in gestation such that heart rate shows little change with hypoxemia. In our study with animals at 0.6 of gestation fetal heart rate was in fact initially increased over the first 2 to 3 hours of sustained hypoxemia, returning to control levels thereafter. Boddy et al.[1] have also reported fetal tachycardia in response to induced hypoxemia in preterm fetuses (100 days' gestation), as have Iwamoto et al.[15] and Gleason et al.,[20] which may relate to a predominance of sympathetic cardiac effects over parasympathetic effects at earlier gestational ages. Near term an increase in fetal arterial blood pressure is a well-described cardiovascular response to hypoxemia mediated through aortic chemoreceptor stimulation and α-adrenergic receptor-induced vasoconstriction.[1, 21] In our study fetal arterial blood pressure remained unchanged throughout the period of sustained hypoxemia as previously reported for the preterm fetus with short-term hypoxemia.[1, 15, 20] This developmental difference in the fetal cardiovascular response to hypoxemia may further relate to a relative immaturity of chemoreceptor function and sympathetic innervation of vascular smooth muscle; this difference may also relate to an inadequate rise in circulating vasoactive substances at earlier gestational ages, as noted in our study and as previously reported by Iwamoto et al.[15]

Fetal breathing activity during normoxic control conditions occurred 22% of the time, which is somewhat less than the time measured in animals near term.[1-5] Although comparable data are not available from other studies at midgestation, developmental changes in fetal breathing activity have been described and are clearly dependent on the means by which this activity is measured. Ioffe et al.[22] reported diaphragmatic electromyographic activity of the ovine fetus at 0.5 to 0.6 of gestation to be primarily tonic in nature with a prolonged burst duration, although periods of phasic diaphragmatic activity were also evident. With advancing gestation phasic diaphragmatic activity became more pronounced in keeping with the increased incidence of fetal breathing movements as measured by tracheal pressure change in animals near term. Bowes et al.[23] reported diaphragmatic electromyographic activity to be almost continuous at the earliest gestation studied (108 days' gestation), although no distinction was made between tonic and phasic activity, with increasing apneic modulation thereafter. Clewlow et al.[2] reported similar findings with the addition that changes in tracheal pressure were often small and difficult to detect in early gestation and were greater when the diaphragm and intercostal muscles were active simultaneously. Conversely, Dawes et al.[24] noted breathing movements to be intermittent in fetal lambs from 40 days' gestation onward delivered into a warm saline bath, and the incidence changed little as measured in chronic preparations with tracheal catheterization from 92 days until term. Although measurement of fetal breathing activity by means of tracheal pressure changes will not reflect all neurally and, in turn, muscularly generated activity of respiratory origin, a summation of this activity should be evident pending the attainment of a threshold level to overcome the resistant properties of the airways. It follows that the lower incidence of fetal breathing activity, here measured at 0.6 of gestation, will in turn reflect either a lower output of respiratory muscle activity or an increase in pulmonary resistant properties at this gestational age. Of interest, the incidence of human fetal breathing movements as measured by real-time ultrasonography is also similarly reduced in the preterm fetus occurring ~14% of the time at 24 to 28 weeks' gestation.[25] In contrast to breathing activity, electroocular and nuchal muscle activity were evident during much of the normoxic control period, a finding also reported by Clewlow et al.[2] for animals at 100 days' gestation and before the differentiation of electrocortic activity and the establishment of well-defined behavioral states.

Fetal breathing and electroocular and nuchal muscle activity, here measured at 0.6 of gestation, showed little change in response to sustained hypoxemia with progressive acidemia in contrast to the well-described decrease in such activity near term when fetal oxygenation is sufficiently compromised.[1-6] Although the degree of fetal hypoxemia and resultant acidemia was more severe in animals we have similarly studied near term,[4] fetal breathing and electroocular activity were nonetheless markedly decreased when measured at arterial Po_2 and pH values comparable with those of the present study. In animals studied at a similar age, Ioffe et al.[22] noted fetal breathing as measured by diaphragmatic electromyographic activity to be decreased with short-term hypoxemia; however, this reduction appeared to involve primarily the tonic rather than the phasic component of this activity. With advancing gestational age and increased prominence of phasic diaphragmatic electromyographic activity, a greater decrease in such activity was noted in response to hypoxemia. Likewise, both Clewlow et al.[2] and Bocking and Harding[3] report the hypoxic-related diminution in fetal breathing to be quantitatively less in younger gestational-aged fetuses (~110 to 120 days). A developmental change in the inhibitory response of biophysical activity to fetal hypoxemia is thus suggested; this change may relate to the maturation of inhibitory neural mechanisms given

the evidence for such within the brain stem at least for fetal breathing activity.[26]

For the ovine fetus near term the alterations in biophysical activity in response to induced hypoxemia appear to be a protective mechanism whereby energy expenditure and thus oxygen requirements are decreased.[7,8] This in turn provides a physiologic basis for the use of biophysical parameters in the determination of fetal health; indeed, such usage has become the mainstay in the day-to-day monitoring of the high-risk obstetric patient. In our study biophysical activity has been measured at 0.6 of gestation and close to the limit for extrauterine survival of the human fetus at a comparable gestational age. The lack of hypoxia-related change in biophysical parameters at this time in spite of the attainment of blood gas and pH levels similar to those in studies near term may indicate that the fetus remote from term requires a greater deterioration in metabolic status, possibly close to death, before such change will be evident. If the human situation is comparable this will obviously impact on the success of preterm antenatal assessment protocols. More importantly, although the oxygen margin of safety would appear to be greater for the preterm fetus, the inability to elicit a protective decrease in muscular activity may ultimately impact survival should fetal oxygenation become sufficiently compromised.

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Intracellular calcium and the signaling mechanism of luteinizing hormone–releasing hormone in rat granulosa cells

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OBJECTIVE: The purpose of these studies was to determine the source(s) of the increase in intracellular free calcium in response to luteinizing hormone–releasing hormone in ovarian granulosa cells.

STUDY DESIGN: Rat granulosa cells were cultured and loaded with fura-2-acetoxy-methyl ester, a fluorescent calcium indicator dye, and intracellular free calcium measured by microspectrofluorometry. The source of the luteinizing hormone–releasing hormone induced increase in intracellular Ca^{++} was investigated with various calcium channel blockers (verapamil, diltiazem, and nifedipine), high K^+ buffer, and perifusion with media lacking Ca^{++}.

RESULTS: All three voltage-sensitive calcium channel blockers (10^{-5} mol/L) tested were ineffective in blocking the luteinizing hormone–releasing hormone induced intracellular Ca^{++} increase. Treatment with high K^+ buffer also had no effect. Perifusion with media lacking calcium resulted in a gradual loss of the luteinizing hormone–releasing hormone response, an effect that was accelerated by repeated stimulation with hormone. Transient replacement of extracellular Ca^{++} failed to restore the response but continued perifusion with Ca^{++}-replete media allowed a luteinizing hormone–releasing hormone response 10 minutes later.

CONCLUSIONS: The luteinizing hormone–releasing hormone induced intracellular Ca^{++} increase does not appear to result from the opening of voltage-sensitive or K^+-dependent Ca^{++} channels. To the contrary, this response likely results from the release of Ca^{++}, primarily from intracellular stores. (Am J Obstet Gynecol 1992;167:541-7.)

Key words: Luteinizing hormone–releasing hormone, granulosa cells, calcium

Luteinizing hormone–releasing hormone (LHRH) is believed to directly affect rat ovarian cells[1-5] by stimulating polyphosphoinositide breakdown in the cell membrane.[6, 7] Inositol 1,4,5-trisphosphate (IP_3) is a product of phosphatidylinositol-4,5-bisphosphate (PIP_2) hydrolysis and has been proposed as a mediator for intracellular Ca^{++} mobilization.[8] Additionally, products of polyphosphoinositide metabolism may also be involved in regulating Ca^{++} entry from the extracellular fluid.[9] With the calcium indicator dye fura-2-acetoxy-methyl ester (fura-2AM) LHRH has been shown to increase intracellular Ca^{++} in single rat granulosa cells.[10] The response to LHRH was rapid and transient. However, it was unclear whether the observed increase in intracellular Ca^{++} was the result of increased entry of Ca^{++} across the cell membrane or enhanced Ca^{++} release from intracellular stores. In our study the contributions of intracellular and extracellular source(s) of Ca^{++} in LHRH-induced intracellular Ca^{++} changes were examined.

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Material and methods

Animals. Female Sprague-Dawley rats (Charles River, Montreal, Canada) were injected with pregnant mare serum gonadotropin (12 IU subcutaneously, day 23) to stimulate formation of preantral follicles. The rats were killed by cervical dislocation after 48 hours, and the ovaries removed.

Preparation of granulosa cells. Granulosa cells were harvested by puncturing the ovarian follicles with a fine hypodermic needle under a dissecting microscope as previously described.[11] The ovaries were squeezed gently in minimum essential medium supplemented with Eagle's salts, 2 mmol/L L-glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, and nonessential amino acids (Gibco, Grand Island, N.Y.). The granulosa cells were recovered by centrifugation for 5 minutes at 200*g*. The cells were washed, resuspended in supplemented Minimum Essential Medium, and plated (0.1×10^6 cells/ml) onto uncoated glass coverslips (18 mm diameter) in 6-well culture dishes and incubated

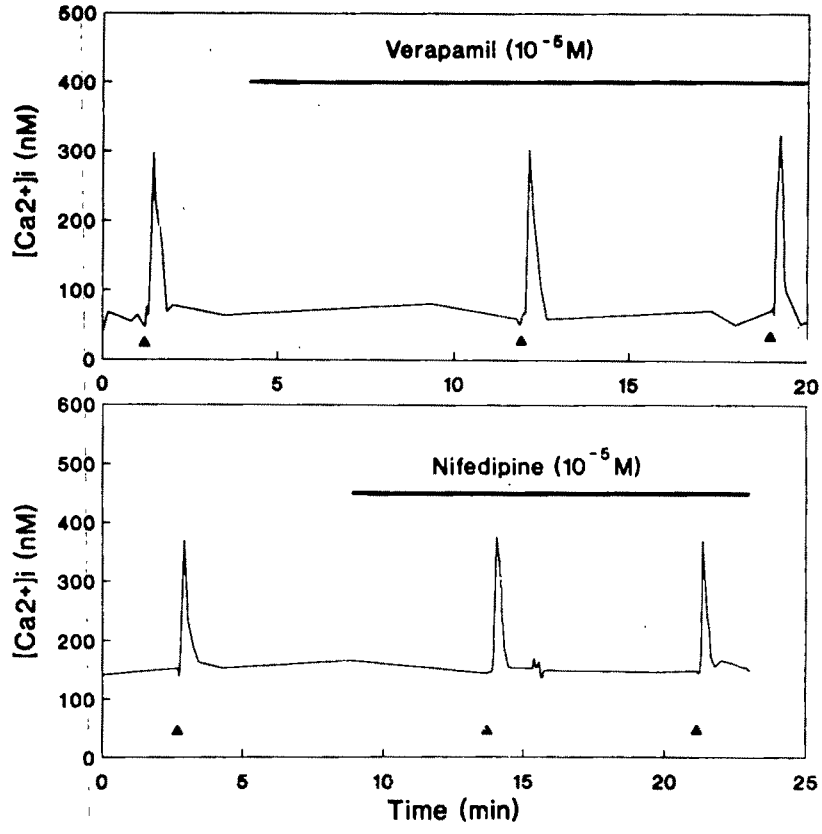


Fig. 1. Effect of voltage-sensitive Ca^{++} channel blockers on LHRH-induced intracellular Ca^{++} increase. LHRH was administered at time indicated by *solid arrow*. Verapamil *(upper panel)* and nifedipine *(lower panel)* were perifused during time indicated by *solid bar* (10^{-5} mol/L, n = 10 for each). Similarly, diltiazem (10^{-5} mol/L, n = 10) had no effect on the intracellular Ca^{++} response to 10^{-6} mol/L LHRH (results not shown).

in supplemented Minimum Essential Medium containing 5% fetal bovine serum. After 48 hours' incubation at 37° C in an atmosphere of 5% carbon dioxide in air, cells were loaded with fura-2AM (Molecular Probes Inc., Eugene, Ore.) as described previously.[10-12]

Fluorescence measurement and calculation of cytosolic calcium. Fluorescence measurement and calculation of cytosolic calcium were reported previously.[10, 12] In brief, individual coverslips were mounted in a laminar flow-through chamber (volume = 350 μl). The chamber was mounted onto the stage of a Zeiss Jenalumar microscope equipped for epifluorescence. The light, from a 200 W mercury arc lamp, first passed through one of two differential interference filters (350 or 380 nm, band widths = 10 nm) that could be rotated by a computer-controlled stepping motor. The light then passed through a 410 nm dichroic mirror, a 100× apochromat oil immersion lens (numerical aperture = 1.4), and an adjustable diaphragm, reducing the light intensity. A field diaphragm was used to reduce the area of illumination to the size of a single granulosa cell. All fluorescent light passed back through the dichroic mirror and a 450 nm band pass filter to reduce background fluorescence. The emitted fluorescence resulting from excitation at 350 nm or 380 nm was deflected either to the eyepiece or to a camera port in which was mounted a photomultiplier tube used to convert the fluorescence into DC voltage. The voltage was converted to a digital form and measurements of fluorescence ratios, corrected for background, were obtained on a 1.8- or 5-second time base.

The method for calculating intracellular Ca^{++} has been reported.[13] A calibration method with the same granulosa cells as described in a previous study[10] was used. An increase of more than 50 nmol/L in intracellular Ca^{++} was considered to be significant.

Treatment regimens. Hormone treatment was added directly to the chamber in a 250 μl volume. LHRH was consistently treated in a concentration of 10^{-6} mol/L. The cells were perifused continuously with Earle's balanced salt solution (EBSS) (or $-Ca^{++}$ EBSS) at a rate of 2 ml/minute. For the Ca^{++} channel studies, cells were perifused with channel blockers (10^{-5} mol/L) in EBSS or high K^+ buffer (50 mmol/L potassium chloride in Minimum Essential Medium) for 5 minutes before LHRH treatment.

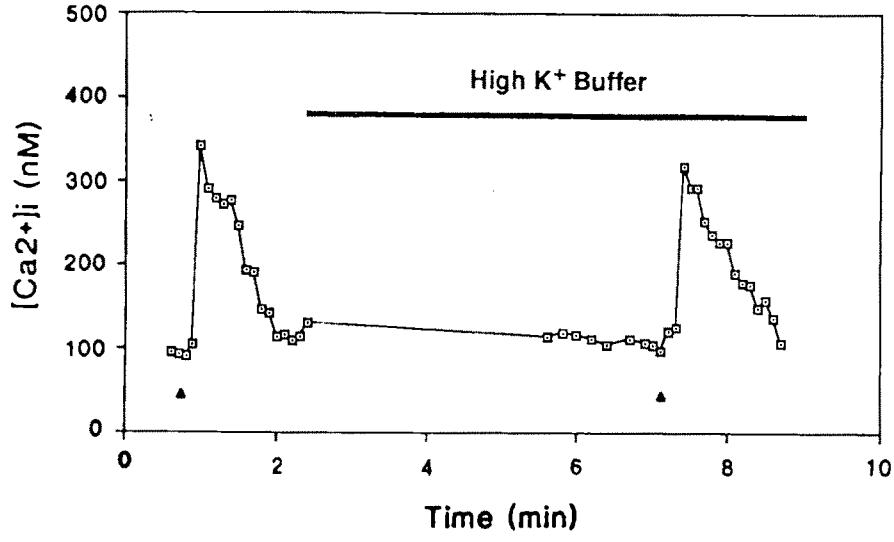


Fig. 2. Effect of high K^+ buffer on LHRH-induced intracellular Ca^{++} increase. Buffer was perifused for the period indicated by *solid bar*. This representative recording illustrates continued responsiveness of cell to LHRH (10^{-6} mol/L) in presence of high K^+ (n = 9). Note expanded scale compared with other Ca^{++} transient recordings.

Reagents. Pregnant mare serum gonadotropin was obtained from the National Institute of Diabetes, Digestive and Kidney Disease and the National Hormone and Pituitary Program (University of Maryland, School of Medicine). LHRH, Ca^{++} channel blockers, and all other reagents were obtained from Sigma (St. Louis).

Results

LHRH-induced transient increase in cytosolic calcium. Two hundred eleven rat granulosa cells from 25 different preparations were treated with LHRH (10^{-6} mol/L). The average resting level of intracellular Ca^{++} in these cells was 98.5 ± 4.8 nmol/L and intracellular Ca^{++} increased by an average of 3.9-fold in the 136 (64%) cells that responded to LHRH. Similar observations have been reported previously.[10]

Effect of calcium channel blockers on LHRH-induced increase in intracellular Ca^{++}. Calcium channel blockers of voltage-sensitive Ca^{++} channels were used in our study to examine the possible involvement of extracellular Ca^{++}. Verapamil (phenylalkylamine-type calcium channel blocker), nifedipine (dihydropyridine-type blocker), and diltiazem (benzothiazepine-type blocker) had no effect on the LHRH-induced increase in intracellular Ca^{++} (10^{-5} mol/L, n = 10 for each). Representative recordings are shown in Fig. 1.

Effect of high K^+ buffer on LHRH-induced intracellular Ca^{++} increase. High K^+ buffer was used to investigate a possible involvement of K^+-dependent Ca^{++} channels in the LHRH-induced intracellular Ca^{++} increase. Perifusion of granulosa cells with high K^+ buffer did not affect the resting intracellular Ca^{++} levels (data not shown). Furthermore, perifusion with this buffer for 5 minutes before LHRH treatment did not affect the LHRH-induced intracellular Ca^{++} response observed during perifusion with EBSS (n = 12). A representative recording of a single granulosa cell is shown in Fig. 2. The apparent difference compared with other Ca^{++} transient recordings is the result of the expanded scale.

Effect of $-Ca^{++}$ EBSS perifusion on LHRH-induced intracellular Ca^{++} increase. In order to investigate the contribution of extracellular Ca^{++} to the LHRH-induced intracellular Ca^{++} increase, the cells were perifused with $-Ca^{++}$ EBSS. Over a period of 8 to 20 minutes there was a gradual decrease and eventual elimination of the intracellular Ca^{++} response to LHRH (n = 10). Repeated stimulation with LHRH in media lacking Ca^{++} resulted in a more rapid loss of the response (i.e., within 13 minutes; Fig. 3, *upper panel*). However, after a recovery period the same cell was able to respond (albeit in an attenuated fashion) to LHRH after 13 minutes of $-Ca^{++}$ EBSS perifusion provided it was not treated with LHRH during this time (Fig. 3, *lower panel*).

After 7 minutes' perifusion with EBSS containing 1.8 mmol/L Ca^{++} the cells again showed a rapid and transient elevation of intracellular Ca^{++} in response to 10^{-6} mol/L LHRH (Fig. 4). A similar response was observed in 14 individual cells.

Effect of transient addition of Ca^{++} to LHRH-induced intracellular Ca^{++} increase. After the initiation of $-Ca^{++}$ EBSS perifusion LHRH-induced intracellular Ca^{++} changes were decreased gradually and eventually abolished at 1, 6, and 16 minutes, respectively (Fig. 5). Subsequently the cells were exposed to four

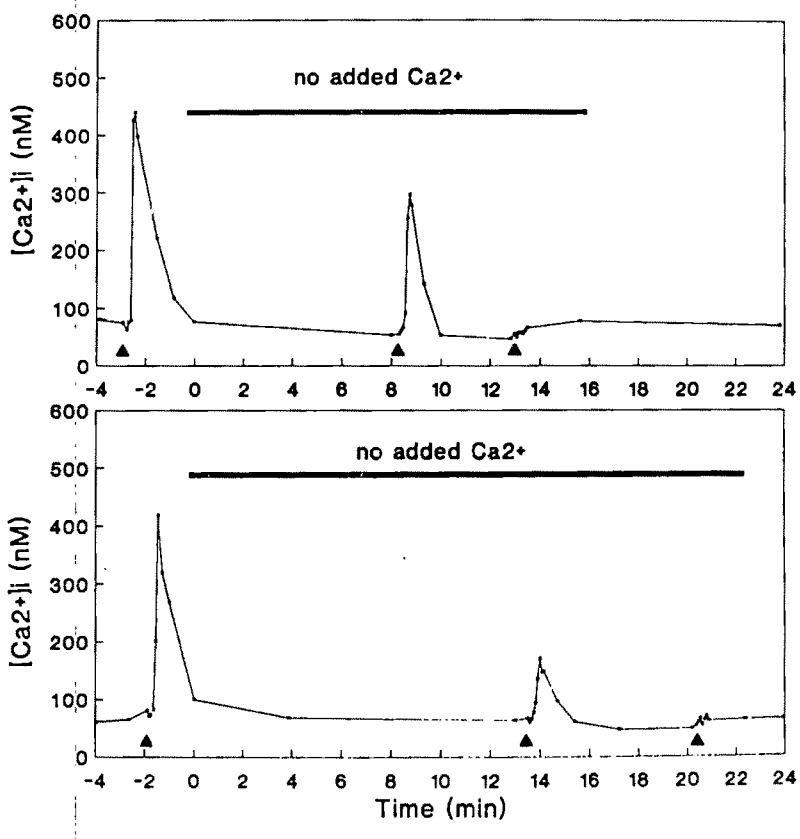


Fig. 3. Effect of $-Ca^{++}$ EBSS perifusion on LHRH-induced intracellular Ca^{++} increase. $-Ca^{++}$ EBSS was perifused for period indicated by *solid bar*. *Upper panel*, cell shows decreased LHRH response at 8 minutes, with complete loss of response at 13 minutes. *Lower panel*, same cell was tested after 10-minute recovery period in normal media. Response to 10^{-6} mol/L LHRH could be elicited after 13 minutes of $-Ca^{++}$ EBSS perifusion provided there was not prior LHRH treatment during this time.

successive injections of LHRH (10^{-6} mol/L) in EBSS containing 2 mmol/L, 5 mmol/L, 10 mmol/L, or 20 mmol/L calcium chloride, respectively. Perifusion of 1 ml of each of these solutions allowed the extracellular calcium concentration to be transiently restored or even increased. There was no observed effect of any of the four treatments on intracellular Ca^{++} ($n = 8$). However, continued perifusion with EBSS containing 1.8 mmol/L Ca^{++} for 10 minutes restored the intracellular Ca^{++} response to LHRH in the same granulosa cell (Fig. 5).

Comment

Previous studies have demonstrated that LHRH stimulates polyphosphoinositide hydrolysis in ovarian granulosa cells.[6,7] Inositol 1,4,5-triphosphate generated during polyphosphoinositide breakdown has been proposed as a mediator of intracellular Ca^{++} mobilization.[8] Inositol lipid metabolism is also believed to be involved in the regulation of Ca^{++} entry from the external environment by inositol 1,3,4,5-triphosphate.[9] In the ovary increases in intracellular Ca^{++} may serve to modulate the stimulatory or inhibitory effects of LHRH on progesterone and prostaglandin E_2 accumulation.[14-16] Calcium is required for the inhibitory action of LHRH on cyclic adenosine monophosphate and steroid production during long-term incubation of ovarian cells.[17,18] In addition, the presence of a Ca^{++} chelator, ethylenediaminetetraacetic acid, in the culture medium can diminish the LHRH-induced increase in progesterone production in short-term cultures.[19,20]

In our study the calcium-sensitive fluorescent indicator fura-2AM was used to study the effect of LHRH on intracellular Ca^{++} in single rat granulosa cells. LHRH caused rapid and transient increases in intracellular Ca^{++}, confirming our previous observation that Ca^{++} participates in the action of LHRH on rat granulosa cells.[10] Again, there did not appear to be a sustained phase of elevation after the transient increase, unlike the intracellular Ca^{++} response to gonadotropin-releasing hormone in pituitary gonadotrophs.[21] However, the precise source of Ca^{++} that contributes to this increase remained to be resolved.

The possible contribution of extracellular Ca^{++} to

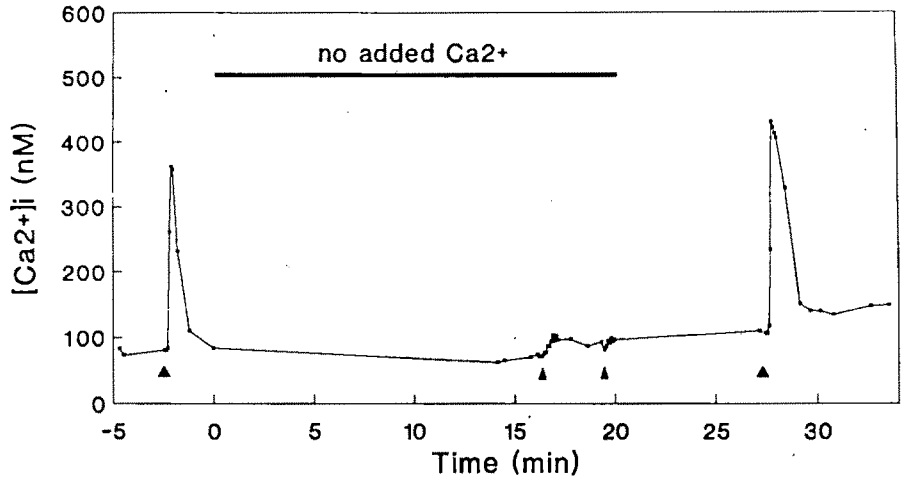


Fig. 4. Replacement of $-Ca^{++}$ EBSS with Ca^{++}-replete media restores LHRH response. Perifusion of $-Ca^{++}$ EBSS was initiated at time zero for period indicated by *solid bar*. Two sequential injections of LHRH (10^{-6} mol/L) 15 minutes later did not generate intracellular Ca^{++} response. However, replacement of 1.8 mmol/L Ca^{++} in perifusion media restored ability of cell to respond to LHRH. Similar results were obtained with 15 cells from seven experiments.

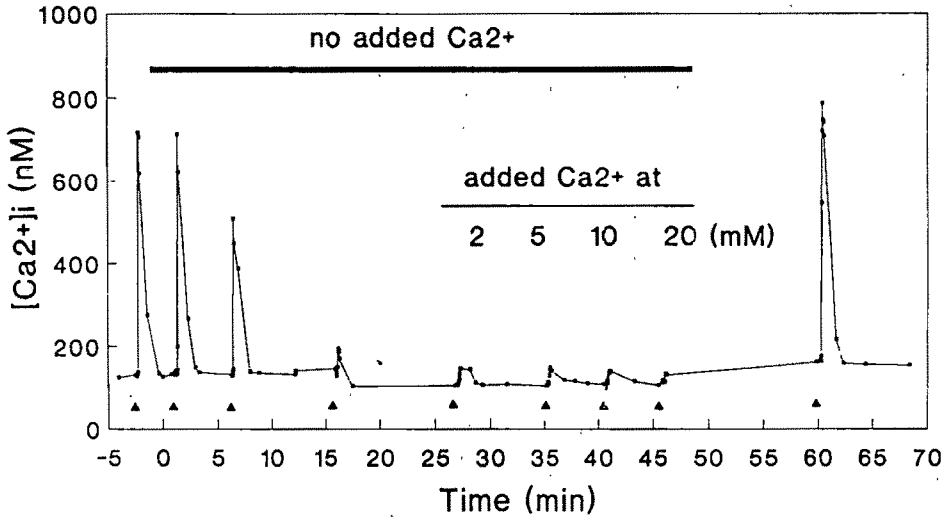


Fig. 5. Transient addition of Ca^{++} to $-Ca^{++}$ EBSS–perifused granulosa cells. Cells which had lost their intracellular Ca^{++} response to LHRH (10^{-6} mol/L) in presence of $-Ca^{++}$ EBSS did not regain responsiveness by transient addition of 2, 5, 10, or 20 mmol/L Ca^{++}. However, continued perifusion with 1.8 mmol/L Ca^{++} in EBSS allowed LHRH response 10 minutes later. $-Ca^{++}$ EBSS perifusion was for period indicated by *solid bar*. Similar observations were made in eight cells from five experiments.

the LHRH-induced intracellular Ca^{++} increase was examined with specific calcium channel blockers. Verapamil, diltiazem, and nifedipine have been shown to effectively block calcium channels at 10^{-5} mol/L.[22-24] However, these blockers had no effect on the LHRH response, suggesting that the hormone does not affect voltage-dependent Ca^{++} channels in granulosa cells. Alternatively, these channels may not exist in this cell type. The presence of both L and T Ca^{++} channels has been demonstrated in chicken granulosa cells with the technique of patch clamping.[25] The L channel was completely inhibited by 16.7 μmol/L nifedipine (dihydropyridine-type calcium channel blocker). It is known that the dihydropyridine type of Ca^{++} channel blocker prevents movement of Ca^{++} through L-type voltage-dependent channels, but is not effective on N- or T-type voltage-dependent channels or on receptor-operated channels.[26] Because verapamil, nifedipine, and diltiazem only influence L-type channels, we cannot dismiss the possibility of LHRH influencing other types of volt-

age-dependent Ca^{++} channels. However, to our knowledge these voltage-dependent channels have not been reported to exist in rat granulosa cells.

The possibility of LHRH effects on K^+-dependent Ca^{++} channels was studied with high K^+ buffer. The lack of effect of high K^+ buffer on the LHRH-induced response indicates that these channels do not contribute to the increase in intracellular Ca^{++}. Furthermore, perifusion with high K^+ buffer did not affect the resting intracellular Ca^{++} level, suggesting that these channels may not even exist in rat granulosa cells.

The involvement of extracellular Ca^{++} in the transient LHRH-induced intracellular Ca^{++} increase was also investigated with media lacking Ca^{++}. LHRH-induced changes in intracellular Ca^{++} were completely abolished by perifusion of the cells with $-Ca^{++}$ EBSS within 20 minutes. This abolition could have been caused by either the lack of an extracellular Ca^{++} source or a depletion of intracellular Ca^{++}. Support for the latter arises from the observation that complete abolishment of the LHRH-induced intracellular Ca^{++} response was a gradual process. Considering the volume of the cell chamber (350 μl) and the flow rate of the perifusion medium (2 ml/minute), an extracellular Ca^{++}-free condition should have been reached in <5 minutes. However, the response to LHRH was still observed (albeit attenuated) for 8 to 14 minutes after perifusion with $-Ca^{++}$ EBSS. Repeated stimulation with LHRH (10^{-6} mol/L) in the same cell resulted in a more rapid loss of the intracellular Ca^{++} response to this hormone under low Ca^{++} conditions (Fig. 3). We suggest that LHRH stimulation accelerated the depletion of intracellular Ca^{++} pools and that the loss of response to LHRH was the result of this effect. Indeed, intracellular stores of calcium normally require repeated stimulation in the absence of external Ca^{++} to be depleted.[27] However, prolonged efficient washing in $-Ca^{++}$ EBSS in the absence of hormone stimulation was also apparently effective in depleting intracellular Ca^{++}, which may suggest the presence of a somewhat more labile LHRH-sensitive pool of intracellular Ca^{++}.

Transient restoration of extracellular Ca^{++} during $-Ca^{++}$ EBSS perifusion failed to restore the ability of the cells to respond to LHRH (Fig. 5). Furthermore, after $-Ca^{++}$ EBSS perifusion, continued perifusion with EBSS-containing 1.8 mmol/L Ca^{++} was required for 5 to 10 minutes before the LHRH-induced intracellular Ca^{++} response was fully restored. The present data indicate that the rapid and transient LHRH-induced intracellular Ca^{++} response results primarily from the release of Ca^{++} from intracellular Ca^{++} pools. However, we cannot dismiss the possibility that extracellular Ca^{++} may be required to maintain these pools in a fashion similar to the "capacitative" model previously described by Putney and Takemura.[28, 29] In this respect extracellular Ca^{++} may play an indirect role in the LHRH-induced release of intracellular Ca^{++}.

Many studies have concentrated on the question of which intracellular pool is responsible for intracellular Ca^{++} mobilization. The site at which inositol 1,4,5-triphosphate acts has been shown to be an adenosine triphosphate–dependent, nonmitochondrial Ca^{++} pool, probably the endoplasmic reticulum.[30] Although the present results indicate that the intracellular Ca^{++} pools are probably responsible for the rapid LHRH-induced increase in intracellular Ca^{++}, the relative importance of the mitochondria and the endoplasmic reticulum in granulosa cell cytosolic Ca^{++} regulation remains uncertain.

Two phases of Ca^{++} mobilization in response to LHRH administration have been seen in pituitary cells.[31-33] The rapid and transient phase has been attributed to Ca^{++} release from intracellular stores, and the latter sustained phase has been attributed to entry of extracellular Ca^{++}.[33-35] We have seen no evidence for this sustained phase in individual rat granulosa cells. However, our observations of the transient intracellular Ca^{++} response to LHRH in the ovarian granulosa cell support the notion of an intracellular source rather than a flux of extracellular Ca^{++} through membrane channels.

In summary, our study shows that LHRH causes a rapid and transient increase in intracellular Ca^{++} in individual rat granulosa cells. Our data suggest that an intracellular pool of calcium is primarily involved in this response. The depletion of this intracellular pool appears to occur simply as a result of prolonged exposure of cells to a medium containing no added calcium. In addition, this depletion can be accelerated by stimulation with LHRH. Finally, our results support the role of LHRH as an autocrine or paracrine mediator in the rat ovary and the role of calcium as a second messenger in the action of LHRH.

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Comparative analysis of normal and growth-retarded placentas with phosphorus nuclear magnetic resonance spectroscopy

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OBJECTIVE: Phosphorus 31 magnetic resonance spectroscopy studies were carried out on placentas from normal vaginal and elective cesarean deliveries without antenatal complications and from pregnancies complicated by intrauterine growth retardation of unknown cause to determine differences.
STUDY DESIGN: Perchloric acid extraction was performed on frozen tissue, and quantitative analysis was carried out for well-resolved resonances representing adenosine triphosphate, sugar phosphate, inorganic phosphorus, diphosphoglycerate, glycerophosphorylethanolamine, and glycerophosphorylcholine.
RESULTS: Adenosine triphosphate levels were highest in the growth-retarded group. There were significantly higher levels of sugar phosphate, diphosphoglycerate, and glycerophosphorylcholine in the placentas of the growth-retarded pregnancies compared with those from normal placentas.
CONCLUSION: These differences may represent a response to hypoxia and an increase in the amount of blood in the placenta. The results demonstrate the utility of nuclear magnetic resonance spectroscopy for studying the pathology of abnormal placentas to gain a better understanding of the pathology and represent early steps toward in vivo spectroscopic studies of the placenta. (AM J OBSTET GYNECOL 1992;167:548-53.)

Key words: Phosphorus 31 nuclear magnetic resonance spectroscopy, human placenta, perchloric acid extraction, intrauterine growth retardation

Intrauterine growth retardation (IUGR) is a complication of pregnancy affecting as many as 4% to 7% of all pregnancies.[1] The causes of fetal growth retardation include maternal hypertension, cigarette and other substance abuse, in utero infection, and chromosomal disorders. In many cases, however, a specific cause is never identified. The placenta, which serves many critical functions for the fetus, is an important organ to study in any attempt to understand the cause of idiopathic IUGR.

Very little is known about the role of the placenta in fetal IUGR. Most studies have focused on pathologic and histologic examinations. These investigations, however, tended to be nonspecific because of the many different causes of IUGR.[2] Other studies on animals have reported a decrease in glucose and amino acid transfer to the fetus.[3-5] Few studies, however, have yet focused on a biochemical analysis of placental metabolic function. Such analysis may be particularly informative in cases where the cause is unidentified.

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Nuclear magnetic resonance (NMR) spectroscopy offers a unique method for studying placental metabolic function. It is a noninvasive technology that simultaneously monitors naturally occurring molecules in biologic tissue. In a previous report[6] we described the phosphorus 31 magnetic resonance spectra of fresh human placental tissues and perchloric acid–extracted tissue. We also detailed the quantitative analysis of adenosine triphosphate and inorganic phosphorus levels in the placental extracts. In the current study we extend our quantitative analysis to other metabolites easily visible in the phosphorus spectra, such as sugar phosphate, diphosphoglycerate, glycerophosphorylethanolamine, and glycerophosphorylcholine. We have focused our analysis on a comparison of placentas from idiopathic growth-retarded pregnancies and normal pregnancies to gain further insights into the cause of the growth retardation.

Material and methods

Five placentas from pregnancies antenatally identified as complicated by IUGR of unknown cause were obtained at the time of delivery by cesarean section. These were confirmed as growth-retarded placentas by neonatal weights <10th percentile with Brenner's growth curve.[7] The mean birth weight was 1253 ± 207 gm at a mean gestational age of 35.3 ± 0.9 weeks confirmed by ultrasonographic examination before 20 weeks. Thorough chart review and patient interview

revealed no specific cause such as hypertension, cigarette or drug abuse, viral illnesses, or prenatal complications, i.e., bleeding. The mean placental weight was 198 ± 32 gm, and there were no gross abnormalities such as infarctions or abruptio placentae seen on inspection. Small pieces of placental tissue were removed from multiple regions on the maternal side by sharp dissection and placed into liquid nitrogen within 1 minute and stored in a −70° C freezer for later analysis. Similarly, placentas from normal, uncomplicated pregnancies obtained from eight vaginal deliveries and eight elective cesarean section deliveries were harvested and frozen in the same fashion. Placentas in all three groups were obtained in the third trimester.

At the time of extraction the tissue was removed from the freezer and pulverized in liquid nitrogen with a stainless steel Waring blender (Dynamics Corp. of America, New Hartford, Conn.). A perchloric acid extract was performed as previously described.[6] Briefly, a 20 gm sample of finely ground placental tissue was mixed with 20 ml of 1 mol/L perchloric acid that had been precooled to −78° C in dry ice. The perchloric acid–tissue mixture was allowed to warm to approximately −10° C in the ambient air for 15 minutes during which time the metabolites were extracted. The extract was then centrifuged at 40,000*g* at −4° C for 20 minutes to separate the solid fraction from the liquid fraction. The liquid supernatant containing the extracted metabolites was then decanted into a chilled beaker and neutralized to pH 7.0 at 4° C through the dropwise addition of potassium hydroxide. The potassium perchlorate precipitated during neutralization was removed by a second centrifugation step identical to the first, and the supernatant was then passed over a Chelex-100 chelating resin (Sigma Chemical Co., St Louis) to remove divalent cations. The extract was again titrated to neutral pH with diluted perchloric acid, frozen in a vacuum flask (Virtis Co., Gardiner, N.Y.), and lyophilized to dryness. The extract was then resuspended in 5 ml of 40% deuterated water for quantitation of spectra. The extracts were placed in the outer chamber of a coaxial 10 mm NMR tube (Wilmad Glass, Buena, N.J.) with an external reference standard of 25 mmol/L hexachlorocyclotriphosphazene mixed with 31 mmol/L chromium acetylacetonate (Aldrich Chemical Inc., Milwaukee)[8] in an inner coaxial insert. Spectra were obtained with a 21 μsec pulse giving a 70° flip angle, an acquisition period of 1.12 seconds, a relaxation delay of 15 seconds (total pulse recycle time 16.12 seconds), and 160 acquisitions.

When phosphorus nuclei have sufficient time to return to their equilibrium state, i.e., fully relaxed state, their peak intensities are proportional to the concentration of the metabolite present. To assess the consequence of the pulse recycle time (acquisition time of 1.12 seconds plus variable delay) on the extent of nuclear relaxation, spectra were obtained at recycle times of 6.12, 11.12, 16.12, 21.12, 31.12, and 61.12 seconds. The hexachlorocyclotriphosphazene and β-adenosine triphosphate resonances are fully relaxed under conditions of our "standard" 16.12-second recycle time.[6] The sugar phosphate, inorganic phosphate, glycerophosphorylethanolamine, and glycerophosphorylcholine resonances, respectively, return to 93.7%, 97.4%, 95.1%, and 98.6% of their equilibrium states at the 16.12-second recycle time. The following "saturation correction factors" are then used to correct the integrated intensities of the signals obtained at 16.12 seconds: adenosine triphosphate 1.00, hexachlorocyclotriphosphazene 1.00; sugar phosphate 1.07, inorganic phosphate 1.03; glycerophosphorylcholine 1.05, and glycerophosphorylethanolamine 1.01 before calculation of metabolite concentrations.

The quantitative analysis is previously reported.[6] Metabolite quantitation was performed with the external reference standard of hexachlorocyclotriphosphazene and chromium acetylacetonate as a spin relaxation agent (Aldrich) in a coaxial capillary system. In brief, the integrated peak area of a phosphorus resonance was divided by the integrated peak area of the hexachlorocyclotriphosphazene reference signal. The ratio for the β-adenosine triphosphate resonance is then used to quantitate the adenosine triphosphate metabolite present with a calibration curve previously constructed for varying known concentrations of adenosine triphosphate. The adenosine triphosphate levels and the β-adenosine triphosphate/hexachlorocyclotriphosphazene ratio are related by the equation $Y = 0.035 + 0.088X$. After calculation of the concentration of adenosine triphosphate, the remaining peak ratios and their corresponding saturation correction factors were then used to determine the final concentration of the other metabolites.

All spectra were obtained on a 300 MHz NMR spectrometer (model GN-300 WB General Electric, Fremont, Calif.) interfaced with a wide-bore superconducting magnet (89 mm bore, 7.0 tesla field) in the Duke Magnetic Resonance Spectroscopy Center. A standard 20 mm broad band probe (General Electric) tuned to 121.4 MHz was used for ^{31}P spectroscopy. Spectroscopic data were collected at the ambient operating probe temperature (approximately 22° C) without temperature regulation. To avoid radiofrequency heating effects on the tissue and extracts, no proton decoupling was used. ^{31}P chemical shifts are referenced to phosphocreatine at 0.00 parts/million.

Statistical analysis was accomplished with a Statgraphics program (Statistical Graphics Corporation, STSC, Rockville, Md.). The means and standard deviation of each metabolite were calculated for the three

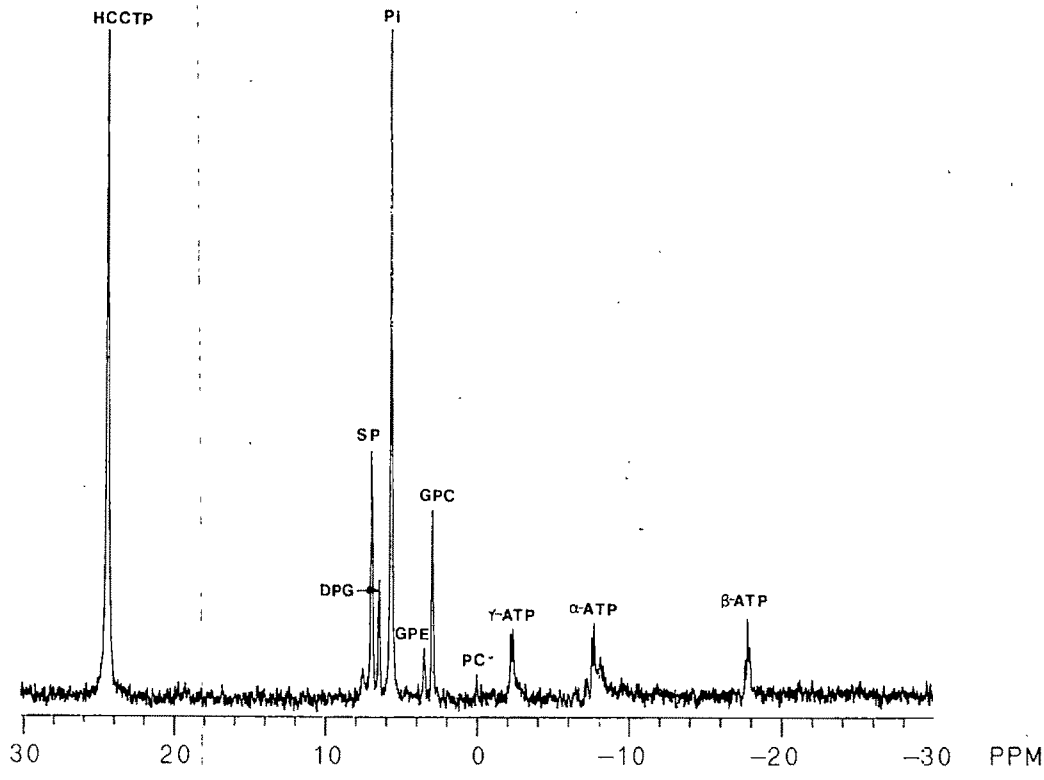


Fig. 1. Phosphorus NMR spectrum of perchloric acid–extracted placental tissue from normal vaginal delivery. Note resonances for external reference standard hexachlorocyclotriphosphazene *(HCCTP)*, diphosphoglycerate *(DPG)*, sugar phosphate *(SP)*, inorganic phosphate *(Pi)*, glycerophosphorylethanolamine *(GPE)*, glycerophosphorylcholine *(GPC)*, phosphocreatine *(PCr)*, and adenosine triphosphate *(ATP)*.

groups (vaginal delivery, cesarean section, and growth retardation). A one-way analysis of variance was performed to determine the presence of a statistical difference among the means of the three groups. A multiple-range analysis or a *t* test was performed to determine specific differences among each of the three groups.

Results

Fig. 1 shows the phosphorus spectrum from a perchloric acid extract of a normal placenta. Of note are the distinct and well resolved resonances for hexachlorocyclotriphosphazene, sugar phosphate, diphosphoglycerate, inorganic phosphorus, glycerophosphorylethanolamine, glycerophosphorylcholine, and the three phosphorus nuclei of adenosine triphosphate. Note that there is very little phosphocreatine at 0 parts/million, and there is a large amount of inorganic phosphorus.

Table I lists the placental metabolite concentrations determined by ^{31}P NMR for vaginal-delivery placentas, cesarean-section placentas and growth-retarded placentas delivered by cesarean section. The table specifically lists the mean concentration and standard deviation for each of the metabolites of the three placenta groups. The mean concentrations of the phosphorus metabolites are highest in the IUGR group, by almost a factor of two compared with the vaginal and cesarean section groups, except for inorganic phosphorus. There are no statistically significant differences among the metabolites between the vaginal and cesarean section placentas. However, there are statistically significant differences among the adenosine triphosphate, sugar phosphate, diphosphoglycerate, and glycerophosphorylcholine levels between the growth-retarded placentas and those from normal vaginal and cesarean section deliveries.

Comment

The current study is a comparative analysis of placentas from normal and abnormal pregnancies with phosphorus NMR spectroscopy. Our findings suggest that NMR spectroscopy, with its ability to simultaneously display the levels of several metabolites, can be extremely useful for in vitro and in vivo biochemical analysis. In this study the NMR analysis yields further insights into placental metabolism. Human in vivo NMR studies in diagnostic medicine are currently focused on ischemic brain injury, muscle dystrophy, and tumor response to chemotherapy.[9] With continued de-

Table I. Placental metabolite concentrations determined by phosphorus NMR spectroscopy

Metabolite	*Normal vaginal delivery (n = 8) (mean ± SD) (μmol/gm wet weight)*	*Normal cesarean section (n = 8) (mean ± SD) (μmol/gm wet weight)*	*Growth retardation (n = 5) (mean ± SD) (μmol/gm wet weight)*	*p Value**
Adenosine triphosphate	0.270 ± 0.189	0.426 ± 0.154	0.547 ± 0.160†	0.0295
Sugar phosphate	1.103 ± 0.308	1.502 ± 0.305‡	2.137 ± 0.319§	0.0001
Inorganic phosphate	2.586 ± 0.308	3.477 ± 1.643	3.720 ± 1.777	0.2740
Diphosphoglycerate	0.383 ± 0.135	0.426 ± 0.151‡	0.712 ± 0.090§	0.0010
Glycerophosphorylethanolamine	0.273 ± 0.095	0.347 ± 0.084	0.440 ± 0.166	0.0528
Glycerophosphorylcholine	0.556 ± 0.139	0.685 ± 0.130‡	1.112 ± 0.348§	0.0005

*The p values obtained from a one-way analysis of variance to test differences between means in all three groups.

†Statistically significant difference between the growth-retarded and normal vaginal delivery groups by a t test but not the multiple range test.

‡Statistically significant difference between the growth-retarded and normal cesarean section groups by multiple range test.

§Statistically significant difference between the growth-retarded and normal vaginal delivery groups by multiple range test.

velopments in technology, detailed analysis may someday be feasible for in vivo spectroscopic studies of the human placenta.

Our current report focuses on differences between spectra from tissue extracted with perchloric acid both for the increased resolution available to identify biochemical change and with the expectation that these differences, once established, may be visible in whole placental tissue. It is an important first step to characterize the NMR spectra of human placental extracts to facilitate the analysis of peaks in the spectra from gross tissue for relevant biochemical and diagnostic studies in the future. Our previous preliminary studies on gross placental tissue[6] demonstrated obvious resonances for sugar phosphate, inorganic phosphorus and adenosine triphosphate. The diphosphoglycerate, glycerophosphorylethanolamine, and glycerophosphorylcholine resonances were not well resolved. In addition, phosphocreatine was absent in the spectrum of gross tissue. However, with perchloric acid extraction we were able to confirm the presence of phosphocreatine albeit in very small quantities. We were also able to determine tissue adenosine triphosphate levels from the extracts.

In this study we performed further analyses on the spectra from perchloric acid–extracted tissue. We found different adenosine triphosphate levels in the three groups studied. They were lowest in the vaginal delivery group, although not statistically different from the cesarean section group. The adenosine triphosphate level is highest in the IUGR group with a statistical difference from the vaginal delivery group. Bloxam[10] also reported lower levels of adenosine triphosphate in placentas from vaginal deliveries than from placentas from cesarean section. This may, in part, be due to varying amounts of blood within the placentas. Less blood is expected in vaginal delivery placentas because blood is squeezed out of the sinuses during labor and delivery. We have previously found[6] that the blood concentration within the placentas is significant and may account for as much as 30% to 50% of the total amount of adenosine triphosphate seen. Therefore the fact that the highest level was found in the IUGR placentas is interesting and suggests that these placentas may have the largest amount of blood. This is further supported by the fact that diphosphoglycerate, which is a major component of blood, is present at the highest level in the IUGR group and is statistically different from the other two groups. This increase in blood content in the IUGR placentas may represent a compensatory increase in blood flow.

It is also possible that differences in the adenosine triphosphate levels result from varying oxidative phosphorylation requirements within the placentas at the time of delivery. Levels are likely to be lower in the vaginal delivery group because the adenosine triphosphate is used during the process of labor. The increased levels in placentas from growth-retarded pregnancies, on the other hand, could arise from a need to increase oxidative phosphorylation to maintain fetal viability because of possible uterine stresses. Although glycolysis predominates in the placenta,[10] the hypothesis that other aerobic pathways in stressed placentas may produce larger amounts of adenosine triphosphate cannot be excluded. The role of adenosine triphosphate in placental tissue has not yet been totally characterized, although it has been shown to be important in the act of ion transport in placental tissue[11] and its levels have been investigated in preeclamptic placentas.[12, 13] Without knowing the actual contribution of blood in an individual placenta we are unable to determine the true increase in oxidative phosphorylation from the tissue per se. To our knowledge there are no accurate means to determine blood content with our methodology, and other means, such as histologic examination of each placenta to compare the intervillus vascular space with the villus tissue space, are at best estimates of the blood contribution.

The most interesting and particularly remarkable finding in this study is the result that the overall phosphorus metabolite levels not restricted to adenosine triphosphate levels alone are higher in the growth-retarded placentas compared with the normal placentas (Table I). On the basis of our analysis there appear to be statistically significant differences in the metabolites of the growth-retarded placentas compared with the others in all categories, except for inorganic phosphorus and for glycerophosphorylethanolamine, where the concentration difference was close to being statistically significant with a p value of 0.0528. A statistically significant difference in the sugar phosphate levels, which includes such compounds as glucose-6-phosphate and fructose-6-phosphate, could represent enhanced carbohydrate metabolism, through glycolysis, in the growth-retarded placentas. The difference in the diphosphoglycerate levels is striking and, as previously mentioned, may be consistent with a higher amount of blood in the placenta. The differences noted in the glycerophosphorylethanolamine and glycerophosphorylcholine levels suggest a higher level of membrane phospholipid synthesis or turnover, because these are important components in cell membrane phospholipids.[14] A previous study by Noyszewski et al.[15] also reported an increase in the glycerophosphorylcholine peak intensity in growth-retarded placentas compared with normal placentas, although their results were not quantitated and were performed on pieces of fresh, nonextracted placenta bathed in a perfusate. In this current study the higher levels of nucleotide, carbohydrate, and membrane components in the growth-retarded placentas may be important indicators of increased cell damage and repair in these placentas, compared with a normal healthy placenta.

The highest levels of metabolites were found in inorganic phosphorus. Although there was no significant difference among the levels in the three groups, it was the highest in the IUGR group, which may be consistent with increased cellular metabolism because it has been suggested that large amounts of inorganic phosphate may be necessary for enzymatic function.[16]

Although the current results do not necessarily explain the cause of the growth retardation in the pregnancies studied, they do suggest that there may be placental response to hypoxia. Fox[17] and MacLennan et al.[18] have shown that human placental villi and trophoblasts in culture undergo syncytial degeneration and a marked increase in the number of cytotrophoblastic cells when exposed to hypoxic conditions. The higher levels of phosphorus-containing metabolites seen in our IUGR placentas could similarly represent repair and regenerative processes in the placenta in response to hypoxia. In these cases of idiopathic IUGR, hypoxia may exist in the placental bed but may not be recognized clinically. A placental reaction to hypoxia in these idiopathic cases of fetal growth retardation is consistent with other conditions known to be associated with IUGR, such as hypertension or infection, because these conditions can lead to hypoxia in the placental bed. Therapeutic efforts directed at correcting the hypoxia, such as with the use of aspirin to decrease thromboxane production,[19] may be further supported by these findings.

Finally, it should be pointed out that the analyses undertaken in this study could have been carried out with other traditional biochemical tools. However, the NMR spectroscopic analyses offers the distinct advantage of a simultaneous study of all phosphorus-containing metabolites in one spectrum and the prospect for using NMR as an in vivo biochemical probe. Our current spectral results, which demonstrate an overall increase in phosphorus metabolites, have allowed us the insight that clinically unrecognized hypoxia may be one of the underlying causes of idiopathic IUGR.

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Electrocortical activity, electroocular activity, and breathing movements in fetal sheep with prolonged and graded hypoxemia

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OBJECTIVE: Our objective was to determine the effect of a prolonged and graded reduction in fetal arterial oxygen saturation on electrocortical activity and associated biophysical variables.
STUDY DESIGN: Fourteen unanesthetized fetal sheep were studied between 126 and 135 days' gestation with continuous monitoring of electrocortical and electroocular activity and breathing movements, during a 24-hour control period, and subsequently during 4 days of prolonged and graded hypoxemia induced by progressively lowering the maternal inspired oxygen concentration.
RESULTS: Graded reduction in fetal arterial oxygen saturation resulted in little change in arterial pH until close to 30% when metabolic acidemia was apparent. The incidence of low-voltage electrocortical activity, electroocular activity, and breathing movements were marginally decreased with hypoxemia alone; however, a significant decrease was not apparent until associated with the onset of fetal acidemia.
CONCLUSION: Hypoxemia of a chronic nature must approach the level at which acidemia becomes apparent before a marked change in fetal behavioral activity is noted. (Am J Obstet Gynecol 1992;167:553-8.)

Key words: Fetal behavior state, breathing movements, hypoxemia

It is well recognized that the human fetus near term displays a periodicity in behavioral and biophysical parameters with coincidence of such into well-defined behavioral states similar to those described for the human newborn.[1] Cyclic changes in fetal behavioral state and state-related parameters are thus characteristic of the healthy fetus and provide a basis for the biophysical assessment of fetal health.[2] However, although biophysical parameters appear to reflect fetal health at the time of study,[3] their time course for alteration when fetal oxygenation becomes restricted is unknown, which may limit their usefulness in predicting fetal compromise.

Studies with the chronically catheterized fetal lamb with short-term, induced hypoxemia show the incidence of low-voltage electrocortical activity, electroocular activity, and breathing movements to be variably reduced.[4-6] Although further supporting the role of biophysical parameters in the assessment of fetal health,

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these studies do not predict the response to a slow and progressive decrease in fetal oxygenation, as might be encountered in the high-risk human pregnancy. To determine the effect of a progressive decrease in fetal oxygenation on behavioral activity, we have therefore studied fetal electrocortical activity as a measure of behavioral state and associated biophysical parameters, including electrooocular activity and breathing movements in the chronically catheterized sheep fetus with prolonged and graded reductions in fetal arterial oxygen saturation.

Material and methods

Surgical procedures. Guidelines for the care and use of animals were followed as established by the Canadian Council on Animal Care and approved by the university. Studies were performed on 14 fetal sheep of mixed breed between 126 and 135 days' gestation (term 147 days). Fetuses were prepared for study at least 3 days before initiation of all experiments. The ewes were anesthetized with 1.0% to 1.5% halothane and 50% oxygen/50% nitrous oxide, and the uterus was exposed and opened through a lower abdominal midline incision. Polyvinyl catheters were placed in the brachiocephalic trunk, the inferior vena cava, and the sagittal sinus, as previously reported.[7] Tracheal and amniotic fluid catheters were also placed as previously described.[7] Electrodes of Teflon-coated stainless steel wire (Cooner, Chatsworth, Calif.) were implanted biparietally on the dura for fetal electroencephalographic recordings and through the lateral orbital ridge of the zygomatic bone of each eye for electrooocular recordings. A reference electrode was placed in the loose connective tissue overlying the occipital bone of the skull. The catheters, containing heparinized saline solution, and wires were exteriorized through the flank of the ewe, and the abdomen was closed in layers. Polyvinyl catheters were then placed in the maternal femoral artery and vein. At the time of surgery, 1 gm of dihydrostreptomycin and 800,000 U of penicillin G were injected intramuscularly into the ewe, and after the uterus was closed 1,000,000 U of penicillin G was injected into both the fetal hind-limb vein and the amniotic cavity. This antibiotic coverage was continued daily for 2 to 3 days. Postoperatively the animals were maintained in individual cages suitable for continuous monitoring, with food and water available.

Physiologic measurements. Each fetal lamb was studied during a 24-hour normoxic control period and subsequently during 4 days of prolonged and graded hypoxemia. Strain gauge manometers (Statham model P-23 ID, Gould, Oxnard, Calif.) and a strip-chart recorder (model 78D, Grass, Quincy, Mass.) were used to record pressures in the trachea, amniotic fluid, and brachiocephalic artery. Mean arterial blood pressure referenced to amniotic fluid pressure was calculated as diastolic plus 0.4 of pulse pressure. Electrical signals were processed with a common mode rejection preamplifier (model 7 P511J, Grass). Fetal heart rate was measured by a cardiotachometer (model 7P44B) triggered from the arterial pulse pressure wave. Fetal electrocortical and electrooocular potentials were displayed directly on the chart recorder passing through a passive band-pass filter, 0.3 to 30 Hz on the preamplifier.

Continuous recordings of electrocortical, electrooocular potentials, tracheal pressure, amniotic fluid pressure, fetal arterial blood pressure, and fetal heart rate were obtained throughout the normoxic control period and the 4 days of induced hypoxemia. During this time the ewe's head was placed in a perspex chamber (volume 4.6 cubic feet) secured within the animals' cage, yet allowing for free access to food and water. Prolonged and graded hypoxemia was induced by progressively lowering the oxygen concentration within the perspex chamber with 1% to 2% carbon dioxide added as monitored by periodic measurement of oxygen and carbon dioxide gas tensions; on day 1 to 18%, day 2 to 16%, day 3 to 12% to 14%, and on day 4 to 10% to 12% with the change in maternal inspired oxygen concentration occurring at approximately 10 AM each day. Total gas flow to the perspex chamber was 40 L/min. Fetal arterial blood was sampled three times each day at ~8 AM, 12 noon, and 3 PM during both the 24-hour control period and the 4 days of induced hypoxia. At 12 noon each day a paired sagittal vein blood sample was also obtained and radionuclide-labeled microspheres were injected into the inferior vena cava for determination of regional organ blood flows.

All blood samples were analyzed for oxygen saturation, hemoglobin, oxygen content, pH, P_{O_2}, and P_{CO_2}. Brachiocephalic artery blood samples at 12 noon each day were also analyzed for corticotropin and cortisol. Fetal blood loss per day was ~4 ml from blood sampling and 4 ml for reference sampling for blood flow determinations. On completion of each daily microsphere injection and subsequent blood sampling, the fetus was transfused with 8 ml maternal blood.

Arterial oxygen saturation and hemoglobin were measured in duplicate by an OSM2 (Radiometer, Copenhagen) hemoximeter, and oxygen content was then calculated with an oxygen capacity of 1.36 ml oxygen/gm hemoglobin[8]. Blood pH, P_{O_2}, and P_{CO_2} were analyzed on an ABL-3 (Radiometer) blood gas analyzer with temperature corrected to 39.5° C. Oxygen and carbon dioxide concentrations within the perspex chamber were also analyzed on an ABL-3 blood gas analyzer.

Data analysis. Although all animals showed a progressive decrease in oxygenation, fetal arterial oxygen saturation values were nonetheless variable, averaging 57.0% ± 2.3% (SEM) on the control day (range 70.4 to 44.2) and with induced hypoxia decreasing to 46.2% ± 3.1% on day 1 (range 63.3 to 27.0), 36.4% ± 3.3% on day 2 (range 55.6 to 18.8),

Table I. Brachiocephalic arterial blood measurements

	Arterial oxygen saturation				
	>60%	*60%-50%*	*50%-40%*	*40%-30%*	*<30%*
Arterial oxygen saturation (%)	65.3 ± 0.9 (n = 13)	54.5 ± 0.8* (n = 13)	46.1 ± 0.7* (n = 12)	35.5 ± 0.6* (n = 13)	22.7 ± 1.0* (n = 14)
Arterial oxygen content (mmol/L)	3.9 ± 0.1 (n = 13)	3.3 ± 0.1* (n = 13)	2.9 ± 0.1* (n = 12)	2.4 ± 0.1* (n = 13)	1.5 ± 0.1* (n = 14)
PaO_2 (torr)	25.7 ± 1.0 (n = 13)	22.2 ± 1.0 (n = 13)	20.6 ± 1.2† (n = 12)	18.4 ± 1.3* (n = 13)	16.4 ± 1.3* (n = 14)
$PaCO_2$ (torr)	45.7 ± 0.7 (n = 13)	45.9 ± 1.0 (n = 13)	45.6 ± 1.3 (n = 12)	46.0 ± 1.4 (n = 13)	48.3 ± 1.7 (n = 14)
pH (arterial)	7.37 ± 0.01 (n = 13)	7.37 ± 0.01 (n = 13)	7.37 ± 0.01 (n = 12)	7.35 ± 0.01 (n = 13)	7.26 ± 0.02* (n = 14)
Base excess (mmol L)	1.1 ± 0.4 (n = 13)	0.9 ± 0.5 (n = 13)	0.7 ± 0.7 (n = 12)	−0.3 ± 0.6 (n = 13)	−5.5 ± 1.5* (n = 14)
Hemoglobin (gm/100 ml)	10.0 ± 0.3 (n = 13)	10.2 ± 0.3 (n = 13)	10.4 ± 0.3 (n = 12)	10.8 ± 0.3 (n = 13)	10.8 ± 0.4 (n = 14)

Data presented as mean ± SEM..
*$p < 0.01$.
†$p < 0.05$.

Table II. Incidence of low- and high-voltage electrocortical activity, electroocular activity, and breathing movements

	Arterial oxygen saturation				
	>60%	*60%-50%*	*50%-40%*	*40%-30%*	*<30%*
Low-voltage electrocortical activity (%)	53.0 ± 1.4 (n = 12)	49.5 ± 2.4 (n = 12)	47.6 ± 1.6 (n = 11)	46.3 ± 2.3 (n = 12)	35.1 ± 4.1* (n = 13)
High-voltage electrocortical activity (%)	40.0 ± 1.7 (n = 12)	45.2 ± 2.6 (n = 12)	46.9 ± 2.8 (n = 11)	46.5 ± 3.0 (n = 12)	58.5 ± 4.8† (n = 13)
Electroocular activity (%)	45.9 ± 3.5 (n = 12)	40.1 ± 4.0 (n = 12)	38.3 ± 2.2 (n = 11)	34.0 ± 4.4 (n = 11)	17.0 ± 4.2* (n = 12)
Fetal breathing movement (%)	27.0 ± 3.4 (n = 10)	20.8 ± 4.5 (n = 9)	19.3 ± 5.7 (n = 7)	19.0 ± 5.0 (n = 9)	5.7 ± 2.6* (n = 10)

Data presented as means ± SEM.
*$p < 0.01$.
†$p < 0.05$.

28.9% ± 3.1% on day 3 (range 43.8 to 15.2), and 22.4% ± 2.4% on day 4 (range 29.6 to 12.8). The time course for this decrease was also variable, with only seven of the 14 animals studied through the entire 4 days of induced hypoxemia, because four of the animals showed a developing metabolic acidosis of the third day and three of the animals on the second day of induced hypoxia with the animals put to death that same day. Results obtained have therefore been grouped according to fetal arterial oxygen saturation with a mean value obtained for each animal within a grouping for multiple values. Data are then presented as grouped means ± SEM in relationship to changes in arterial oxygen saturation. Measurements were not obtained from all animals for each oxygen saturation grouping because of experimental variability in the response to induced oxygen concentration changes within the perspex chamber and to intermittent catheter or electrode failure (Tables I and II). Electrocortical activity was assessed by visual analysis into periods of high voltage (100 to 200 μV), low voltage (<50 μV), and intermediate voltage (50 to 100 μV). Fetal breathing movements were defined as repeated negative deflections in tracheal pressure (corrected for amniotic pressure) of >2 mm Hg lasting for >30 seconds. The incidence of electrocortical states, electroocular activity, and fetal breathing movements were determined as a percentage of the recording time evident for the hour before and after each fetal blood sampling with the measured arterial oxygen saturation presumed to be representative of the fetal blood gas status for that time interval. The data reported were examined by nonpaired t test analysis with significance determined with a Bonferroni correction for multiple comparisons. Cerebral metabolic data and the regional blood flow changes will be reported separately as will the corticotropin and cortisol data.

Results

The oxygen concentration in the perspex chamber measured 21.6% ± 0.3% on the control day and with induced hypoxia decreased to 17.6% ± 0.3% on day

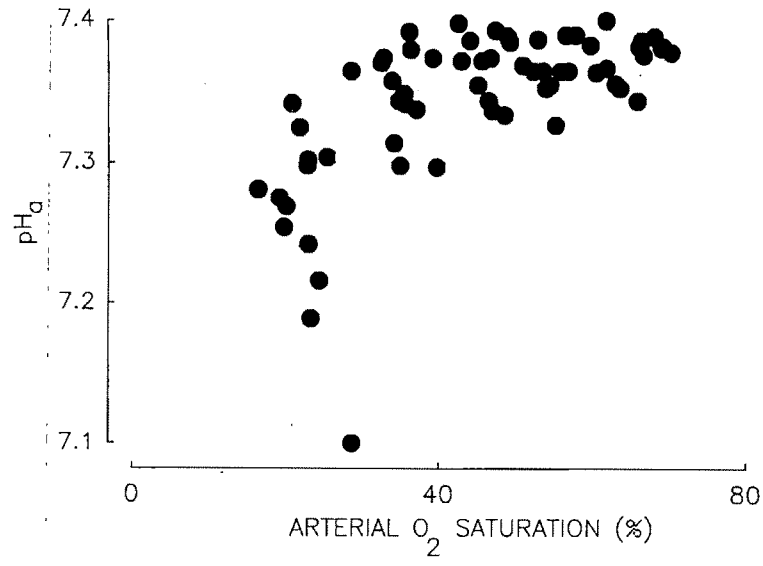


Fig. 1. Grouped and meaned arterial pH values for individual animals plotted in relationship to arterial oxygen saturation. Progressive reduction in fetal oxygenation resulted in little change in arterial pH until arterial oxygen saturation was close to 30%, when onset of metabolic acidosis was evident.

1, 15.7% ± 0.4% on day 2, 12.9% ± 0.5% on day 3, and 11.3% ± 0.5% on day 4. The corresponding carbon dioxide concentrations within the chamber were 0.9% ± 0.1% on the control day and then 1.9% ± 0.1% on day 1, 2.1% ± 0.1% on day 2, 2.5% ± 0.2% on day 3, and 2.9% ± 0.2% on day 4. The animals appeared well and continued to eat and take water. Fetal brachiocephalic arterial blood measurements from the control and study days are shown in Table I, grouped according to induced changes in fetal arterial oxygen saturation. With the graded fall in arterial oxygen saturation, arterial oxygen content and P_{O_2} also fell, whereas arterial P_{CO_2} remained little changed until terminally, when P_{CO_2} did rise in three of the fetuses. Arterial pH also remained little changed until arterial oxygen saturation was between 30% and 40%, when three of the animals began to show a developing metabolic acidosis with a related fall in base excess (Fig. 1). With arterial oxygen saturation <30% all animals except one showed a metabolic acidosis with variable falls in arterial pH noted. Fetal hemoglobin concentration showed no significant change throughout the study.

The incidence of low- and high-voltage electrocortical activity, electroocular activity, and breathing movements from the control and study days are shown in Table II, again grouped according to induced changes in fetal arterial oxygen saturation. The percent time spent in low-voltage electrocortical activity averaged 52.0 ± 1.4, with fetal arterial oxygen saturation >60%, and showed a marginal decrease with oxygen saturation between 30% and 60%. Even with fetal arterial oxygen saturation <30% and a metabolic acidosis now apparent in most animals, low-voltage electrocortical activity, although significantly decreased, was still evident 35.1% ± 4.1% of the time on average. This decrease in the incidence of the low-voltage electrocortical state with graded hypoxemia was mainly due to an increase in the incidence of the high-voltage state, because the percentage time spent in the intermediate-voltage state remained little changed throughout the study and that state was evident approximately 5% of the time on average.

The incidence of electroocular activity, which occurred almost exclusively during the low-voltage electrocortical state, was also marginally decreased with oxygen saturation between 30% and 60%; however, a marked and significant decrease was not noted until arterial saturation was <30%, with electroocular activity now occurring only 17.0% ± 4.2% of the time ($p < 0.01$) (Table II). The incidence of fetal breathing movement, which also occurred almost exclusively during the low-voltage state, was likewise marginally decreased with oxygen saturation between 30% and 60%. However, a marked and significant decrease was again not evident until oxygen saturation was <30% with fetal breathing movements now occurring only 5.7% ± 2.6% of the time ($p < 0.01$) (Table II).

Comment

In this study we have determined the change in fetal behavioral state and associated biophysical parameters in response to a progressive lowering of fetal oxygenation induced by a stepwise decrease in maternal inspired oxygen concentration. The fetal arterial oxygen saturation of all animals measured >50% at some time

point on the control day and showed a subsequent decrease to <30% over the study period. In spite of the progressive decrease in oxygenation, fetal arterial oxygen saturation values were quite variable between animals for a given day of study, as was the time course for the decrease in oxygenation. The data have therefore been analyzed according to the induced change in fetal arterial oxygen saturation rather than the day of study, to better define the relationship of fetal behavioral activity to blood gas change. Although "rate of change effects" will not be apparent with such an analysis, all animals were exposed to the same stepwise decrease in maternal inspired oxygen concentration, resulting in a graded reduction in fetal arterial oxygen saturation with the day-to-day drop no greater than 20% for any of the animals studied.

With the progressive fall in fetal arterial oxygen saturation, arterial oxygen content and partial pressure of oxygen also fell, although to a lesser extent, presumably due to the small increase in hemoglobin concentration over the course of study and the rightward shifting of the oxyhemoglobin dissociation curve with the terminal onset of metabolic acidosis. The graded reduction in arterial oxygen saturation resulted in little change in arterial pH until close to 30%, when a metabolic acidosis was apparent in most animals. At this time arterial oxygen saturation was decreased to approximately half that of normoxic control values, which averaged 62.0% ± 1.1% for all animals. Although fetal oxygen delivery (the product of umbilical venous oxygen content and umbilical blood flow) was not measured, a similar decrease would be anticipated, given the expected linear relationship between oxygen delivery and circulating oxygen levels with this animal model of induced hypoxemia.[9] This finding of metabolic acidemia as graded reductions in arterial saturation approach 50% of normal is thus similar to that with acute reductions in fetal oxygen delivery, where metabolic acidosis is only evident with reductions >50%.[9]

In this study low-voltage electrocortical activity was evident 53% of the time on average with fetal arterial oxygen saturation >60%, which is similar to that previously reported[4-6] for normoxic control study at a comparable gestational age. A marginal decrease in this activity was noted with arterial oxygen saturation between 30% and 60%. Although not statistically significant, this marginal decrease may nonetheless be biologically important and represent a protective mechanism whereby the fetus is able to change to a metabolic state with lower oxidative needs, at least for the brain, as we have previously shown cerebral oxidative metabolism to be increased during the low-voltage electrocortical state.[10] With arterial oxygen saturation <30%, the incidence of the low-voltage electrocortical state was significantly decreased ($p < 0.01$), now occurring 35% of the time, although cycling of low- and high-voltage electrocortical activity was still evident. At this time a metabolic acidosis was noted in all animals except one. The findings of the current study are thus similar to those with short-term reductions in fetal oxygenation,[4-6] where a reduction in the low-voltage electrocortical state is only seen with pronounced hypoxemia, usually in association with metabolic acidemia. However, it is unlikely that acidemia per se resulted in the reduced incidence of the low-voltage electrocortical state, because induced central nervous system acidosis by perfusion of the cerebrospinal fluid results in little change of electrocortical state.[11] It would seem rather that hypoxemia is the stimulus for the alteration in electrocortical state but must be of sufficient degree and therefore in most instances associated with acidosis before a significant change in electrocortical activity is noted, given the cyclic nature of this activity.

The incidence of electroocular activity and breathing movements was also marginally decreased with fetal arterial oxygen saturation between 30% and 60%. This may be accounted for by the decrease in the low-voltage electrocortical state, because the reductions were similar. However, a marked and significant decrease in this biophysical activity was again not evident until arterial oxygen saturation was <30%. At this time electroocular activity was decreased to approximately 40% and breathing movements to approximately 20% of that seen with arterial oxygen saturation >60%. This decrease in the incidence of fetal biophysical activity with pronoucned hypoxemia is considerably greater than that seen for the low-voltage electrocortical state (at approximately 65% of well-oxygenated values), suggesting differing mechanisms for their reduction. Alternatively, the same mechanism causes inhibition of biophysical and low-voltage electrocortical activity but with less effect on the latter. Additionally, fetal breathing movements would appear to be a more sensitive biophysical indicator for pronounced hypoxemia than would electroocular activity, at least under the current study conditions.

Previous studies in the ovine fetus with short-term reductions in oxygenation suggest that hypoxemia alone can result in a significant decrease in both electroocular activity and breathing movements.[6] However, it has become apparent that this decrease in biophysical activity in response to moderate hypoxemia shows adaptation over several hours with a return to normoxic levels in spite of the persistence of the hypoxemic insult.[12, 13] It is thus not surprising in the current study with graded reductions in fetal oxygenation over 3 to 4 days that fetal biophysical activity was only marginally decreased with hypoxemia alone. It would again seem that when hypoxemia is slowly induced it must be pronounced and thus often associated with acidosis before a significant decrease in fetal biophysical activity is noted.

Although the biophysical response to acutely induced hypoxemia has been well studied in the ovine fetus, information is limited on chronic reductions in fetal oxygenation. Worthington et al.[14] have studied the effects of reduction of placental size in sheep with resultant asymmetric intrauterine growth restriction and chronic hypoxemia without acidemia. Of interest, the incidence of fetal breathing movements was decreased to approximately 20%, a value similar to that of the current study with hypoxemia alone. This decrease in fetal breathing activity with induced intrauterine growth retardation was significant but involved 24-hour periods of observation, which would limit the effects of other influencing factors, including the diurnal nature and cyclicity of breathing activity. A progression from decreased fetal breathing activity to prolonged apnea to abnormal breathing activity has also been reported in sheep before death in utero.[15, 16] Although abnormal breathing patterns were usually only seen with associated acidemia, decreased breathing activity or apnea were evident with hypoxemia alone, as in the current study, supporting hypoxemia as the stimulus for the alteration in behavioral activity rather than any associated acidotic change. Of note, abnormal breathing patterns were not observed in the current study, suggesting these to be terminal events of short duration.

With prolonged and graded reductions in oxygenation, the ovine fetus thus demonstrates chronic hypoxemia but with little disturbance in arterial pH until oxygen saturation approaches 30%, when metabolic acidosis becomes apparent. Likewise, the human fetus with intrauterine growth restriction demonstrates variable hypoxemic change[17, 18] but is usually born without significant acidosis.[19, 20] Hypoxemia alone has a marginal effect on fetal behavioral activity that nonetheless may be of biologic significance and represent a protective mechanism whereby metabolic costs (including those of growth) are decreased over time. However, it would appear that hypoxemia of a chronic nature must approach the level at which acidemia becomes apparent before a marked change in fetal behavioral activity is noted. It is thus not surprising that the behavioral activity of the human fetus with intrauterine growth restriction is often reported to be normal and or only marginally changed.[19-21] As such, fetal biophysical assessment should be seen as a marker for moderate to severe hypoxemic change with heightened surveillance required for the extremely high-risk pregnancy, because the time course for biophysical alteration before fetal death may be relatively short.

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An immunochemical and immunocytologic study of the increase in myometrial gap junctions (and connexin 43) in rats and humans during pregnancy

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Myometrial gap junctions are thought to play a necessary role in control of parturition. In this study we evaluated the presence of gap junctions in myometrial tissues with a specific antibody to the gap junction protein, connexin 43, with immunocytochemistry and immunoblots. Immunofluorescence of rat heart showed gap junctions were localized along the intercalated disks, but no change occurred during pregnancy. Fluorescent staining of rat myometria showed punctate staining of gap junctions between muscle cells during term and preterm birth, illustrating the tremendous increase in gap junctions during labor. Similarly, staining of human myometrial tissues was present in specimens from pregnant patients but absent in tissues from nonpregnant women. Immunoblots of myometrial tissues from rat and humans confirmed the existence of high levels of connexin 43 during term and preterm labor in rats and also during late pregnancy in humans. (Am J Obstet Gynecol 1992;167:559-67.)

Key words: Gap junctions, myometrium, uterus, connexins, parturition, smooth muscle

Gap junctions are the structural entities that allow direct cell-to-cell communication between opposing cells in various tissues.[1, 2] Both electrical and metabolic transmission of signals occur through these specialized membrane regions.[3] Contractions of the myometrium and also the heart are dependent on the underlying propagation of action potentials from pacemaker regions.[4-7] Thus the pathways for coupling between individual small muscle cells are extremely important in coordinating contractility.

In the myometrium of animals electron microscopic studies have demonstrated that the gap junctions are infrequent in nonpregnant cycling states and most of gestation.[8] However, at term and in some cases preterm the gap junction numbers and sizes increase to provide the basis for propagation of electric activity and synchronized contractility during labor.[8] Similar studies of human myometria, taken during either cesarean section or hysterectomy, demonstrate comparable changes, although the junctions are much more difficult to quantify by electron microscopy due to the larger sizes of the muscle cells.[8, 9]

Recently several studies have shown that the gap junctions exist as a homologous group of proteins that form the intercellular channels of the gap junction.[10-13] These proteins are expressed in varying amounts in different cell types. In the heart and rat myometrium a 43 kd protein, termed connexin 43, has been identified as the gap junction protein.[12-14] Some studies have used antibodies to connexin 43 in the analysis of gap junctions in rat myometrium after estrogen treatment and during pregnancy.[14, 15] However, no study has shown data from animals delivering at term or preterm when gap junctions as identified by electron microscopy[8] are known to be at their highest levels, and no study has demonstrated connexin 43 levels in the human uterus.

In this paper we used antipeptide antibodies directed to the carboxy terminal domain of the cardiac protein connexin 43 to investigate the expression of this protein in rat heart and myometrium and human myometrium by immunohistochemical and immunoblotting techniques. This study shows that connexin 43 levels are elevated in the rat myometrium during term and preterm birth. In addition, connexin 43 protein is present in human myometrial tissues taken from pregnant patients.

Material and methods

Animals and tissue processing. Wistar rats were used for all the studies. Nonpregnant animals at in-

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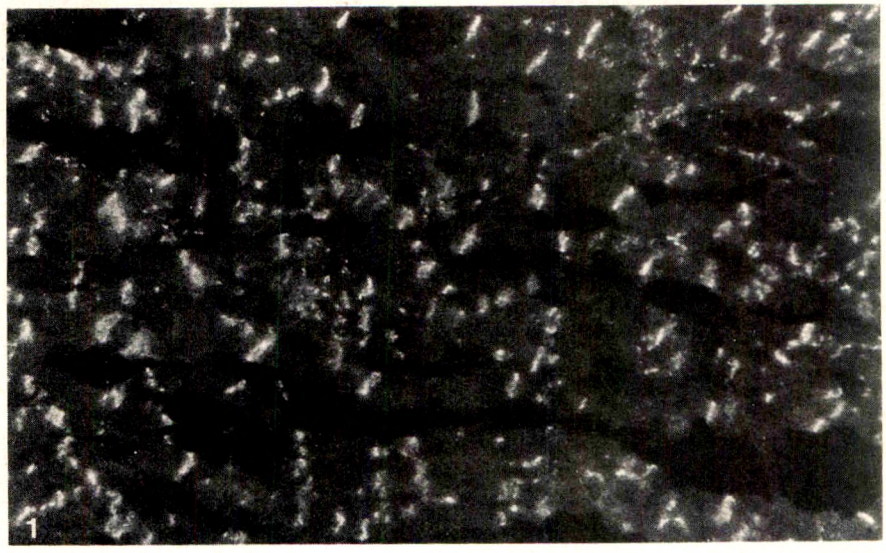


Fig. 1. Low-magnification light photomicrograph of rat heart with epifluorescence for fluorescein isothiocyanate staining with connexin 43 antibody to demonstrate gap junctions. Note muscle fibers positioned horizontally with bright fluorescence concentrated between myocardial cells along intercalated disks. (Original magnification ×380.)

determinant stages of the estrus cycle and timed pregnant rats (day 22 = day of delivery) at various times of pregnancy were used in this study. Tissues were removed from delivering rats after expulsion of two fetuses.

Rats were killed by a blow to the head, and the uterus was removed. The center of each uterus (about 1 cm^2) was pinned on dental wax and rapidly frozen in liquid nitrogen for immunohistochemistry. The remaining pieces were plunged in liquid nitrogen for preparation of microsomal fractions for immunoblotting. Rat cardiac tissues were obtained from the ventricles of the heart and handled in the same manner as uterine tissues.

The human tissue was removed at the time of elective cesarean section and at the time of hysterectomy for benign disease. This study was approved by the institutional review board and consent from each patient was obtained. During cesarean section a 2 × 1 × 1 cm strip was taken from the superior aspect of the uterine incision. Similarly, at the time of hysterectomy of nonpregnant premenopausal subjects, a piece of myometrium was removed from the fundus. The specimens were washed in ice-cold saline solution on collection and then immediately frozen in liquid nitrogen. Small portions of all tissues were processed for immunocytochemical studies and the remainder used for immunoblotting studies.

Some rats were also treated with the antiprogesterone compound RU 486 (Roussel Uclaf, Romainville, France). Timed pregnant rats were injected subcutaneously on day 16 of gestation with 10 mg of RU 486 in sesame oil. On either day 17 or 18 (between 24 and 48 hours after RU 486 treatment) of gestation during premature delivery the rats were killed and the uteruses processed. Control animals were injected with the vehicle only and subsequently treated the same.

Polyclonal antibody to connexin 43. The polyclonal antibody, raised in rabbits, that was used routinely in these studies was directed against the cytoplasmic C terminal domain (amino acids 346 through 360) of connexin 43. The antibody is similar to that used by others,[2, 14-16] and it has been previously shown by immunocytochemistry, immunoblotting, and electron microscopy to bind to gap junctions and connexin 43 protein in the heart.[17]

Immunocytochemical staining. All tissues for immunocytochemical staining were rapidly frozen in liquid nitrogen and stored at −70° C until sectioning. The microscope slides on which the sections were to be placed were coated with poly-L-lysine (10 mg/ml water) and allowed to dry for 30 minutes. The coated slides were then placed in a cryostat at −20° C. The tissue to be sectioned was mounted on a microtome chuck with ornithine carbamyl transferase embedding medium. The tissue was sectioned at 7 to 8 μm thickness. Sections of rat heart were placed on each slide to validate the staining procedure, because the rat heart consistently stains along the intercalated disks with the connexin 43 antibody (Figs. 1 through 3). The sections were allowed to air dry for 1 hour at room temperature. The sections were then rinsed in two changes of phosphate-buffered saline solution at room temperature and then incubated in a blocking solution consisting of 3% bovine serum

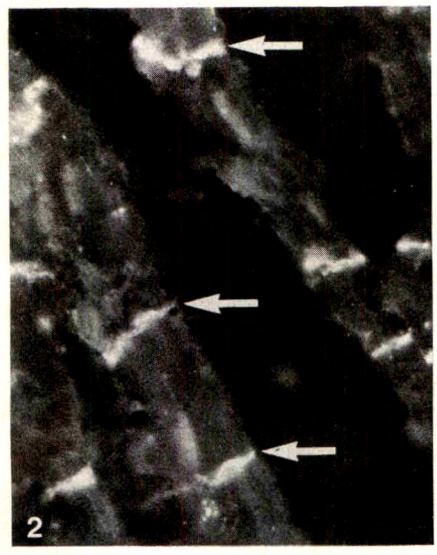


Fig. 2. Higher magnification photomicrograph of heart muscle cells as shown in Fig. 1. *Arrows*, Region of labeling with connexin 43 antibody between cells at intercalated disk area. (Original magnification ×780.)

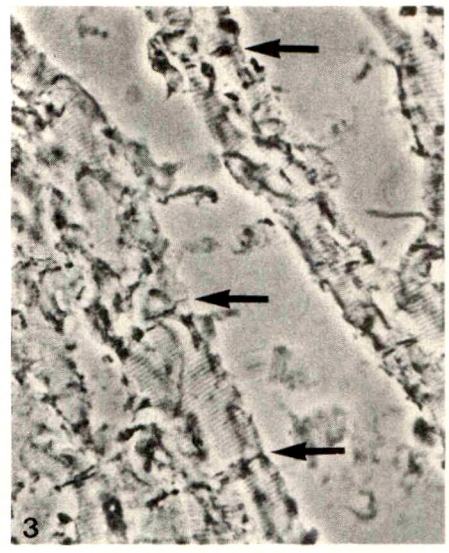


Fig. 3. Phase-contrast photomicrograph of same field as shown in Fig. 2. *Arrows*, Intercalated disks indicated in Fig. 2. Note characteristic striated appearance of muscle cells due to arrangement of myofilaments. (Original magnification ×780.)

albumin and 1% Triton-X100 in phosphate-buffered saline solution for 20 minutes at room temperature. Incubations were carried out in a humidified chamber. The sections were then rinsed in phosphate-buffered saline solution for 10 minutes (two times) at room temperature. The primary antibody (1:250 dilution with phosphate-buffered saline solution) against connexin 43 was placed on the tissue sections, which were then incubated in a humidified chamber overnight at 4° C. The next day the slides were rinsed in phosphate-buffered saline solution (6 × 10 minutes each) and then a fluorescein isothiocyanate–labeled secondary antibody (goat antirabbit immunoglobulin G) purchased from Jackson Immunoresearch Laboratories was added to the sections (1:200 dilution) and the preparation incubated for 1 hour at room temperature. Afterward, the sections were again rinsed in phosphate-buffered saline solution (6 × 10 minutes each), and then 1 to 2 drops of mounting medium (0.4% *p*-phenylenediamine in 0.1 mol/L sodium carbonate buffer, pH 9, diluted 1:1 with 100% glycerol) was placed on each slide and a coverslip applied. The sections were examined with a Zeiss light microscope (magnification ×40 to ×100) with epifluorescence and filters for maximum fluorescein isothiocyanate fluorescence. Table I shows the number of animals and sections examined by immunocytochemistry. We routinely examined negative controls such as sections of heart and myometrium after application of only the secondary antibody to demonstrate the lack of nonspecific staining. Similarly, we occasionally processed liver tissue as above and observed no specific connexin 43 staining.

Table I. Tissues examined with immunocytochemistry

Tissue	*No. of subjects*	*No. of tissues examined*
Rat myometrium		
Delivering	11	36
Day 18, day 19	4	12
RU486, day 17, day 18	4	6
1 Day post partum	3	6
Rat heart	14	158
Human myometrium		
Nonpregnant	4	7
Term		
Labor	7	16
Nonlabor	15	25

Number of animals or patients and the number of tissues examined by immunocytochemistry for connexin 43 content. Rat tissues were obtained during term delivery (delivering), day 18 or day 19 of gestation and at day 17 or day 18 after RU 486 treatment during preterm delivery.

Preparation of crude microsomal fraction. The methods used to obtain a crude microsomal fraction containing the plasma membrane proteins are similar to those used previously.[18] The myometrial tissues were minced with scissors and suspended in six volumes of sucrose-3-(*N*-morpholino)propanesulfonic acid buffer (250 mmol/L sucrose, 20 mmol/L 3-(*N*-morpholino)propanesulfonic acid adjusted with sodium hydroxide to pH 7.0) and homogenized with a polytron PT20 giving three bursts of 10 second duration each at 15,000 revolutions/min. The sucrose-3-(*N*-morpholino)propanesulfonic acid also contained phenylmethylsulfonyl fluoride (1 mmol/L), dithiothreitol (2

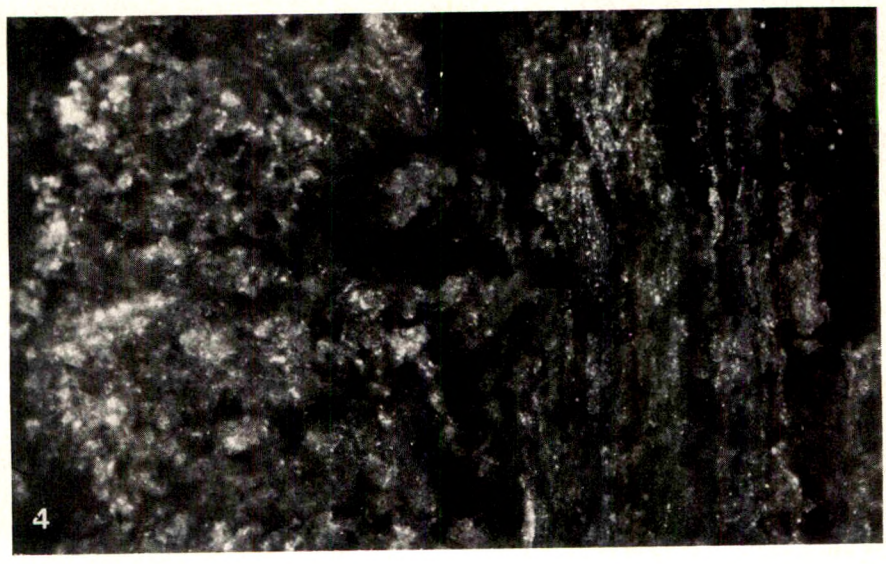


Fig. 4. Photomicrograph of uterine tissue from term delivering rat showing longitudinal (cut in cross section, *left*) and circular (cut in longitudinal orientation, *right*) muscle layers. Bright, punctate staining of gap junctions with connexin 43 antibody is evident in both muscle layers. (Original magnification ×370.)

mmol/L), and trypsin inhibitor (1 mg/ml). The homogenates obtained were centrifuged at 10,000*g* for 10 minutes in a JA20 rotor to yield a pellet and a supernatant fraction. The supernatant was recentrifuged at 115,000*g* for 30 minutes in a Beckman L5-65 ultracentrifuge (with a 60TI rotor) to give a 115,000*g* pellet and a supernatant fraction. This 115,000*g* pellet was resuspended in 8% sucrose. This was the crude microsomal fraction used for the immunoblotting studies. The pellet from the 10,000*g* centrifugation was resuspended in 8% sucrose to check for the presence of connexin 43 with immunoblots. Similarly, the supernatant fraction from the 115,000*g* centrifugation was also analyzed for connexin 43. There were only trace amounts of connexin 43 in the 10,000*g* resuspended pellet and the 115,000g supernatant fraction. Thus the microsomal fraction was shown to contain the majority of the connexin 43 protein. Similar procedures were used for cardiac tissues. Protein concentrations were determined on all fractions according to the method of Lowry et al.[19]

Electrophoresis. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis was carried out with a 12% polyacrylamide separating gel and a 4% stacking gel. The technique used was that according to Laemmli.[20] Before the samples were placed on the gel for electrophoresis, they were heated to 95° C for 5 minutes. The molecular weight markers used were bovine serum albumin (66,000 molecular weight), ovalbumin (45,000 molecular weight), and carbonic anhydrase (29,000 molecular weight).

Immunoblotting. Crude microsomal fractions isolated from rat and human myometrium were resolved in the sodium dodecyl sulfate gels and electrophoretically transferred to nitrocellulose in a BioRad (BioRad, Richmond, Calif.) apparatus at 100 V for 1 hour. The nitrocellulose was incubated with the specific anticonnexin 43 antibody and subsequently reacted with an iodine 125–labeled secondary antibody (goat antirabbit immunoglobulin G, purchased from New England Nuclear, Boston). The nitrocellulose sheets were then autoradiographed with Kodak XAR-5 (Eastman Kodak Co., Rochester, N.Y.) film. After autoradiography was completed (about 3 days), specific areas of the nitrocellulose sheets corresponding to the labeled protein-antibody complex were excised, and the radioactivity in each immunoblot was counted in a γ counter.

Immunoblots were used to determine the amount of myometrial gap junction protein present throughout gestation in the rat. Crude microsomal fractions from day 18, day 22 (delivering), and 20 to 24 hours post partum were prepared and immunoblotted. Fifty micrograms of total microsomal protein from different days of gestation were added to separate lanes of the electrophoresis gel. A preparation of rat heart (also 50 μg) was also run on each gel to act as a positive control. Counts of the radioactivity (counts/min) were used to estimate a ratio between myometrium and heart (counts/min/50 μg protein of uterine tissue ÷ counts/min/50 μg protein of rat heart) to compare the amount of myometrial gap junction protein at different gestational times. The heart tissues used as positive controls were all from rats at midgestation to minimize the possible variation at different times. However, analysis of connexin 43 levels in the heart throughout gestation revealed no significant changes.



Fig. 5. Photomicrograph, similar to those shown in Figs. 1 through 4, of myometrial tissue from rat at 18 days of gestation. Note absence of any punctate type staining as shown in Figs. 4 and 6. (Original magnification ×925.)



Fig. 6. Myometrial tissue from rat at 18 days of gestation during premature birth after treatment with RU 486. Note abundance of staining of gap junctions as compared with tissue from control animals shown in Fig. 5. (Original magnification ×925.).

Statistics. Statistical differences between mean values of various groups of data were determined by one-way analysis of variance. For comparisons of mean values between two groups, the Wilcoxon rank-sum test was used.

Results

Immunohistochemical studies. The anti-connexin 43 antibody specifically stained the intercalated disk regions of the rat heart on sections placed on all slides. Figs. 1, 2, and 3 show rat heart tissues at low (Fig. 1) and high magnification (Figs. 2 and 3). Fig. 3 is a phase-contrast image of the same fluorescent field shown in Fig. 2 illustrating that the bright fluorescent labeling is along the intercalated disk area of the striated heart cells. Findings identical to those above were found in many heart tissue samples (n = 28) from animals at various stages of gestation (Table I). There were no obvious differences in the immunofluorescent staining pattern in hearts of pregnant or nonpregnant animals.

Immunofluorescent staining of rat myometria are shown in Figs. 4, 5, and 6. The pattern of antibody staining in the longitudinal and circular muscle during delivery at term is illustrated in Fig. 4. Bright fluorescent punctate spots can be seen throughout both circular and longitudinal muscle layers. In contrast, there was little or no staining of tissues from myometrial tissues from animals at day 18 or 19 of pregnancy (Fig. 5). However, in tissues from animals delivering prematurely on day 18 of gestation after treatment with RU 486 there were enormous amounts of staining (Fig. 6), and the appearance was like that found in tissues from animals delivering spontaneously at term (Fig. 4). Tissues from numerous animals (Table I) indicated that the connexin 43 staining was elevated only during term and RU 486–induced preterm delivery.

Immunofluorescent staining of human myometrial tissues are shown in Figs. 7 and 8. In tissues from nonpregnant patients (Table I), there was little staining (Fig. 7), and thus the fluorescence was equivalent to that in the rat myometrium before delivery (Fig. 5). Tissues taken from patients at term, both labor and nonlabor groups, showed variable staining with some demonstrating high levels of fluorescence (Fig. 8) as in the rat during delivery (Figs. 4 and 6) and others exhibiting reduced amounts.

Immunoblotting. The antibody (antipeptide 346 to 360) used in the immunoblots exhibited homology with respect to the rat heart and myometrial tissues studied. When 25, 50, and 100 μg of microsomal proteins from both rat and human myometrium were added to different lanes, concentration dependent staining of the immunoblots was observed with distinct bands at about 43 kd. In some cases each band was composed of triplets.

To validate that the antibody was recognizing the connexin 43 protein in a concentration-dependent manner, different protein concentrations of the crude microsomal fraction were quantitatively analyzed after immunoblotting. Plots of the amount of membrane protein added for electrophoresis versus the amount of radioactivity (counts per minute) in the immunoblots showed linear relationships for tissues from rat heart and delivering rat myometrium and also human myo-

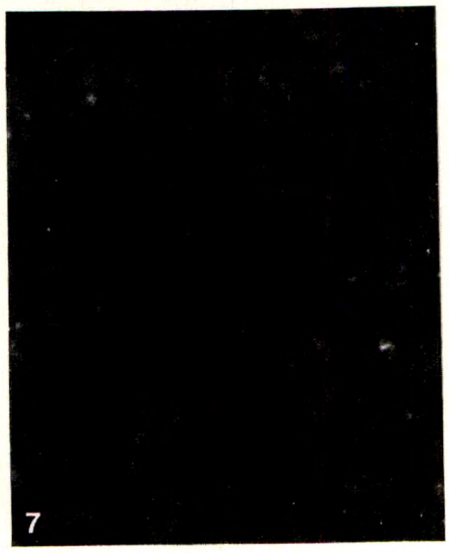


Fig. 7. Human myometrial tissue from nonpregnant patient. Note almost complete lack of connexin 43 staining as compared with myometrial tissues from rat during term (Fig. 4) and preterm (Fig. 6) delivery and also human myometrium from pregnant term patient (Fig. 8). (Original magnification ×1400.)

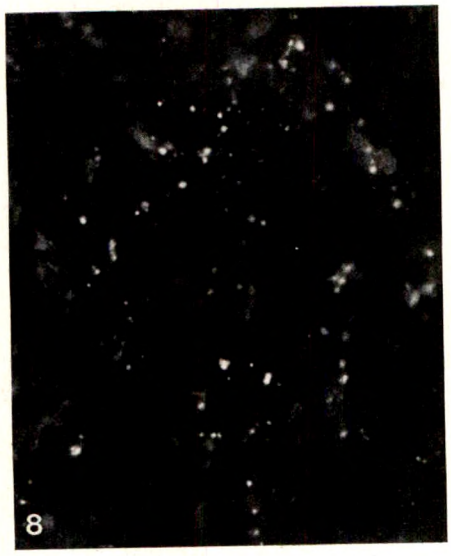


Fig. 8. Human myometrial tissue from term pregnant patient in active labor. Note abundance of staining of gap junctions with connexin 43 antibody. (Original magnification ×1400.)

metrium (Fig. 9). However, there was more radioactivity in immunoblots of preparations from rat heart and myometrial tissues than from human myometria.

Fig. 10 shows immunoblots of heart and myometria from nonpregnant and pregnant rats and humans. There was little 43 kd protein present in tissues from nonpregnant and day 18 pregnant rats compared with tissues from animals that were delivering spontaneously on day 22. Similarly, in tissues from the human uterus there was little 43 kd protein in tissues from nonpregnant patients but elevated levels during pregnancy taken from women either in labor or undergoing elective section (nonlabor). Fig. 11 shows immunoblots of rat heart and myometrial tissues taken from animals at day 18 of gestation without treatment (control) and following RU 486 injections during preterm birth. The blots were much more intense from six animals treated with RU 486 than from control animals. Again, the blots were composed of three separate bands.

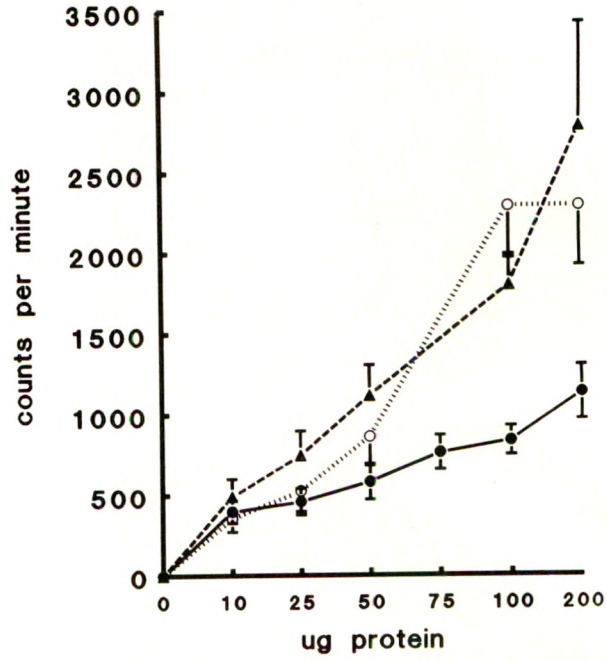


Fig. 9. Concentration dependency of methods used for analysis of immunoblots. Shown are counts per minute of blots versus amount of protein added for electrophoresis for tissues from rat heart (▲) and myometrium (○) and human myometrium (●).

Fig. 12 shows the relative amounts (ratio of connexin 43 levels, uterus/heart) of connexin 43 proteins in rat myometria at different times of pregnancy and during term and preterm birth. There was about three times as much connexin 43 protein present in the myometrium of the delivering rat uterus during spontaneous delivery as present at either day 17 of gestation or post partum. Similarly, animals delivering prematurely after RU 486 treatment showed markedly elevated levels of connexin 43. In animals treated with RU 486 and delivering prematurely, there was an approximately sixfold increase in connexin 43 as compared with day 17 of gestation.

Comment

In this study we have demonstrated through immunohistochemical and immunoblotting techniques the presence of connexin 43 in both rat and human myometrium and also have shown that there is an increase in the protein during term and preterm birth in

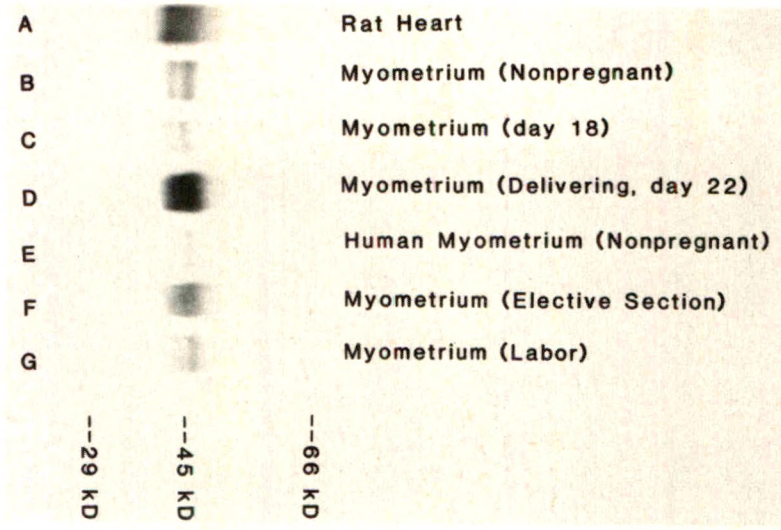


Fig. 10. Immunoblots (50 μg protein each lane) of rat heart and myometrial samples from rats (nonpregnant, day 18 of gestation, and during term delivery) and humans (nonpregnant, elective nonlabor, and labor patients).

the rat. The immunohistochemical studies clearly show the presence of the gap junction protein in the rat myometrium, which peaks during delivery. It has been previously shown with electron microscopy that gap junctions are present in low frequency and small sizes in nonpregnant and preterm and postpartum rats, with an abrupt increase in both size and frequency during delivery.[8, 9] This observation is confirmed and extended with the immunohistochemical studies presented in this study showing minimal staining in the sections at day 18 of gestation, marked staining of the section of delivering tissues, and little staining of the postpartum tissues. Similar results were obtained by measuring the levels of connexin 43 in immunoblots at different times in pregnancy. In this study a crude microsomal fraction was used for the immunoblotting technique. This preparation contains plasma membrane and cytoplasmic components reflecting not only the plasma membrane gap junctions but also the junctions during assembly and degradation. Studies were also conducted showing that this preparation gave the strongest and clearest signal for immunoblotting, far greater than the 10,000*g* pellet or 115,000*g* supernatant fractions.

The use of RU 486, which causes premature labor and increases gap junctions in the rat myometrium as detected by electron microscopy,[21] also results in an increase in connexin 43 protein. The immunofluorescent staining of tissues prepared from RU 486–treated rats appear identical to the sections from term delivering animals with respect to the amount of fluorescence. Correspondingly, the immunoblots showed that tissues from RU 486–treated rats and from term delivering rats both demonstrate high levels of connexin 43, although the former exhibited higher levels. Therefore in the event of premature labor the presence of connexin 43 appears to be the same as that of normal labor, and this confirms that the presence of gap junction is a required step in premature labor.

Each one of the bright fluorescent dots in the myometrial tissues (Figs. 4, 6, and 8) represents one gap junction between muscle cells, approximately 250 nm in diameter. The gap junctions fluoresce with great intensity because each is composed of hundreds of connexin proteins.[1, 22] However, small gap junctions may not be visible with this technique. Furthermore, because sections used in this study are relatively thick (7 to 8 μm and optimum for frozen tissues), any given area could contain many gap junctions (i.e., up to 32 throughout the section depth). It is readily apparent that the junctions are dispersed throughout the tissue and appear to be randomly positioned between smooth muscle cells with no particular region showing higher concentrations. However, this is not the case for heart cells, where the gap junctions are superimposed on each other in the section to give the image of a large unified structure in the region of the intercalated disk. That gap junctions are present in this region in heart muscle has been demonstrated by many.[13, 22]

The immunoblot studies presented support the immunohistochemical results and also provide other information. Quantities of the connexin 43 protein were estimated as normalized to heart tissue. Normalization helped minimize any error associated with the immunoblot technique. This is the first description of a quantitative analysis of the level of connexin 43 in the myometrium during gestation and term and preterm delivery in the rat. Counts of various concentrations of protein show linear relationships indicating that the blots can be used for quantitative evaluation of tissue levels of connexin 43 (Fig. 9). Although the peak values of connexin levels were present during delivery, the immunoblots generally have relatively high levels of

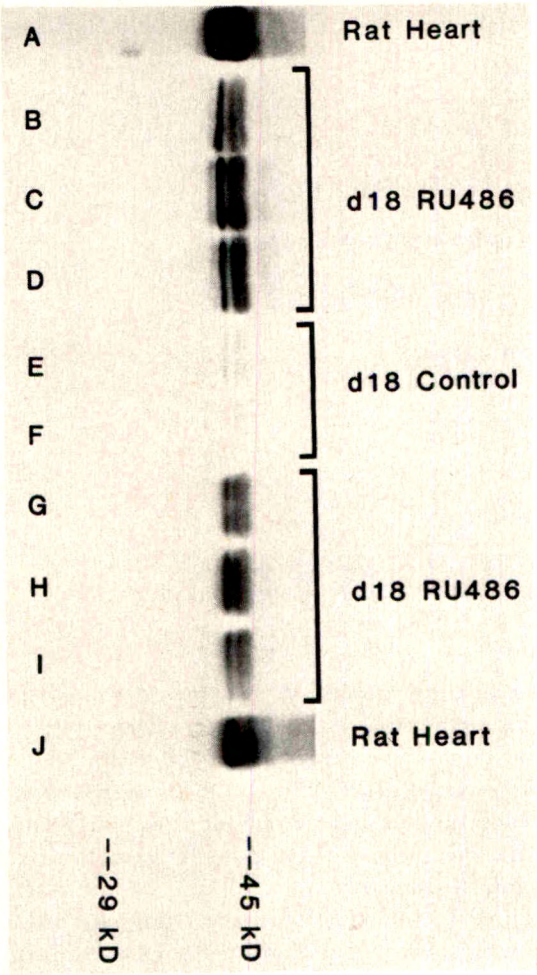


Fig. 11. Immunoblots (50 μg protein each lane) of rat heart *(lanes A, J)* and tissues from six different rats delivering prematurely at day 18 of gestation *(lanes B, C, D* and *G, H, I)* compared with tissues from two control rats at day 18 of gestation *(lanes E* and *F)*. Note distinct division of each blot into three regions.

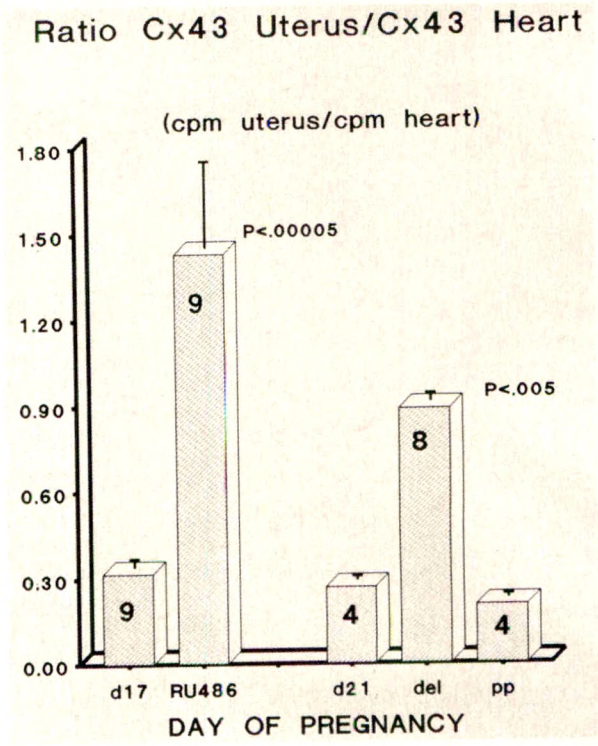


Fig. 12. Histograms showing counts per minute from immunoblots at day 17 of gestation, during delivery, post partum, and after treatment with RU 486 during preterm birth.

connexin 43 protein in tissues from rats and humans (Figs. 10 and 11) before labor and post partum. This is in contrast to previous studies with electron microscopy, which demonstrates few plasma membrane gap junctions between myometrial cells before labor and elevations in junctions during term and preterm labor.[8] Possible reasons for this discrepancy in the rat are (1) presence of binding to gap junctions in other cells besides myometrial cells, (2) presence of small but undetectable gap junctions with both electron microscopy and immunocytochemistry, and (3) binding to gap junction proteins in the cytoplasm of the myometrial cells. The last two possibilities seem the most likely because no visible binding was observed with other cells with immunocytochemistry (no. 1 above). Thus this study is useful in defining the quantity of connexin 43 proteins in the uterus at various times of pregnancy. However, this study does not clarify the exact position of the protein within or between myometrial cells. These studies are only crude approximations of myometrial gap junctions. Studies with the electron microscope still remain the method of choice in defining the presence of gap junctions between myometrial cells.

The regulation of the gap junction protein is not well understood at either the level of transcription or translation. The expression of the transcript in the rat myometrium for connexin 43 has been investigated during gestation with an approximately fivefold increase in transcript levels noted immediately before delivery.[15] This increase was suggested to be the result, in part, of a hormone-induced increase of transcriptional production rate. Posttranslational control may be through phosphorylation of the connexin 43 protein. Although it has been suggested that phosphorylation may play a role in assembly of gap junctions,[23] the possibility of a role in regulation of channel activity or aspects of connexin 43 turnover will require further study.

The observation (Fig. 11) that the anti–connexin 43 antibodies bind to multiple bands on Western blots is consistent with several studies in which it has been shown that they correspond to different phosphorylated forms of connexin 43.[23-25] Roughly equal amounts of the faster migrating unphosphorylated form of connexin 43 (apparent mobility of about 41 kd) and slower migrating phosphorylated forms (doublet with apparent mobility of about 43 kd) are present in our myo-

metrial samples. Although there are significant differences in the relative proportions of the phosphorylated and unphosphorylated forms of connexin 43 in different tissues and cell types, the basis for what must have a physiologic role is not yet understood.

With respect to human samples, this is also the first study to demonstrate the presence of connexin 43 in the myometrium. Our study suggests that there is homology between connexin 43 in the rat myometrium and heart and the human uterus. Both immunohistochemical staining (Fig. 8) and the immunoblots (Fig. 10) clearly show the presence of connexin 43 in pregnant human myometrium. The protein was present in low amounts in the nonpregnant myometrium (Fig. 10). Of particular interest is that connexin 43 was identified in myometrium from patients who had not undergone labor and those who did. The exact quantitative difference, if any, is presently being investigated. It should be noted that all human myometrial tissues from pregnant patients were from the lower uterine segment. Similar electron microscopic studies suggest an increase in human myometrial gap junctions at term.[8] However, in the present studies there was great variability between samples with both immunocytochemistry and immunoblots. Frequently there was greater staining of sections and larger blots from tissues of nonlabor patients than tissues of labor patients (Fig. 10). This may indicate that muscle from the lower uterine segment is not representative of muscle throughout the uterus during pregnancy. The tissues obtained from nonpregnant patients were all from the upper fundal and muscular portion of the uterus. Thus these tissues may not be comparable to those obtained from the lower segment of pregnant patients. In this study the nonpregnant tissues showed little connexin 43 by immunocytochemistry or in the Western blots (Figs. 7 and 10). The human nonpregnant myometrium appears to contain few gap junctions, as has been shown previously by electron microscopy for human myometrium and also in studies of nonpregnant rats.[9] Additional investigations are required to determine the presence of connexins other than connexin 43 in the human nonpregnant and pregnant myometrium.

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LETTERS TO THE EDITORS

Subtherapeutic doses of magnesium sulfate do not inhibit preterm labor

To the Editors: I was disturbed by the article by Cox et al. (Cox SM, Sherman ML, Leveno KJ. Randomized investigation of magnesium sulfate for prevention of preterm birth. Am J Obstet Gynecol 1990;163:767-72.) The authors defined clinically safe magnesium concentrations as "those <8 mEq/L" and attempted to determine whether these levels of magnesium were effective in preventing preterm birth.

The design of the study appears to be inadequate in that the amount of drug administered (4 gm magnesium sulfate bolus and 2 gm per hour infusion) has been shown to be associated with subtherapeutic serum levels of magnesium. Hollander et al.[1] found that the median dosage of magnesium sulfate necessary to achieve tocolysis was 4.5 gm per hour; the mean serum magnesium level to achieve tocolysis was 6.6 mg/dl (5.5 mEq/L). Elliott[2] found serum magnesium levels of 5.1 mg/dl (4.25 mEq/L) with dosages of 2 gm per hour infusion of magnesium sulfate and 6.4 mg/dl (5.3 mEq/L) when 3 gm per hour were given. Somehow in this study the authors state that a maximum magnesium concentration of 5.5 mEq/L was achieved (page 771) or a mean magnesium concentration of 5.5 mEq/L was achieved (page 769). Which is it?

The authors state that the magnesium sulfate was discontinued in eight patients (11%) because of toxicity, mainly generalized muscle weakness (6 patients). Were these patients removed from the study or treated as failures of the drug? To stop the drug because of lethargy and then continue to include these patients in an evaluation of efficacy is not appropriate. Cox states, "The experience of these investigators [Hollander et al.] suggests that effective uterine contraction–inhibiting concentrations of magnesium must be near toxic levels to anticipate a clinical uterine effect." To the contrary, Hollander et al. state in their conclusion, "The drug appears to offer a high therapeutic-to-toxic ratio with a serum concentration response that is easy to determine." Discontinuing the infusion in at least six patients is prejudicial to the study and merely reflects inexperience with the use of the drug.

The authors also fail to mention how many patients received a second course of magnesium sulfate after the initial 24 hours. It is unrealistic to treat a patient in true premature labor for 24 hours with subtherapeutic doses of a drug and then discontinue it.

The bias of the investigators is readily apparent in the discussion, in which they selectively cite articles by Steer and Petrie, Spisso et al., Elliott, and Hollander et al. and essentially dismiss them. They cite the study by Cotton et al. and their own study as the only placebo-controlled randomized studies. In the study of Cotton et al. the dosage of magnesium was also inadequate (4 gm bolus and 2 gm per hour intravenous infusion—no option to raise the infusion rate). The number of patients treated with magnesium sulfate in that study was also totally inadequate—8 patients with *intact* membranes had magnesium sulfate versus 9 saline and 12 terbutaline. The magnesium sulfate was also administered for 12 hours and then stopped.

Apparently the authors ignored other studies (Ferguson et al., Wilkins et al.) in the literature comparing magnesium sulfate with intravenous β-sympathomimetic therapy and finding magnesium sulfate either superior to or comparable with the β-sympathomimetic drugs.

The conclusion reached in the article by Cox et al. that "magnesium sulfate infusions resulting in maximum serum concentrations of magnesium equal to 5.5 mEq/L are ineffective in the prevention of preterm birth" is absolutely true. If their goal was to avoid toxicity they certainly achieved it, but in their zeal to avoid toxicity they also fell far short of therapeutic levels of magnesium in the sera of their patients.

The only conclusion that can be drawn from these data is that magnesium sulfate in subtherapeutic dosages does not inhibit preterm labor better than intravenous hydration.

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Reply

To the Editors: We take it that Elliott did not like our report concerning the lack of efficacy for intravenous magnesium sulfate in the prevention of preterm birth. Elliott has liberally referred to his own 1983 retrospective review of tocolysis with magnesium sulfate,[1] and we are compelled to do so, too.

Elliott reported in 1983 that he administered magnesium sulfate as follows: "After a 4 gm intravenous bolus of magnesium sulfate, a constant infusion was started at 2 gm/hr . . . ." The maximum infusion rate apparently used was 3 gm per hour. A total of 355 pregnancies were included in Elliott's report; 673 magnesium levels were measured subsequent to therapy for an average of 1.9 levels per pregnancy. Interestingly, only 28 magnesium levels were obtained during 3 gm per hour infusions. Does this mean that Elliott's only experience with the higher infusion rate is from approximately 14 pregnancies representing 4% of his study population? Alternatively, Elliott reported that 536 magnesium levels were obtained during 2 gm per hour infusions. Does this mean that 282 (or 79%) of Elliott's patients received 2 gm per hour infusions? Now

Elliott refers to our study and claims, "The design of the study appears to be inadequate in that the amount of drug administered (4g magnesium sulfate bolus and 2 gm per hour infusion) has been shown to be associated with subtherapeutic serum levels of magnesium." We must conclude that Elliott is far more guilty of subtherapeutic therapy than we because we used the higher infusion dosage more often than he did. Specifically, 62% of our patients received 3 gm per hour infusions. Moreover, we are mystified that Elliott claims tocolytic efficacy for magnesium sulfate when most of his patients apparently were treated with what he now calls a subtherapeutic dosage regimen.

Elliott also reported in 1983, "Clinically, we found that levels between 5.0 and 7.0 mg/dl were usually sufficient to inhibit contractions. . . ."[1] We reported a mean magnesium level of 6.6 mg/dl. Elliott now states that "The conclusion reached in the article by Cox et al. that 'magnesium sulfate infusions resulting in maximum serum concentrations of magnesium equal to 6.6 mg/dl are ineffective in the prevention of preterm birth' is *absolutely true* (emphasis added)." In an attempt to apply some logic to all this we seem unavoidably compelled to conclude that Elliott now believes his 1983 statement ". . . levels between 5.0 and 7.0 mg/dl were usually sufficient . . . "[1] is untrue. Our real bias is that Elliott unwittingly seems to agree with us, although we take it he would not like this, either.

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The effect of gestational age on maternal serum α-fetoprotein levels in pregnancies with Down syndrome

To the Editors: Greenberg et al. (Greenberg F, del Junco D, Weyland B, et al. The effect of gestational age on the detection rate of Down syndrome by maternal serum α-fetoprotein screening. Am J Obstet Gynecol 1991;165:1391-3) found that maternal serum α-fetoprotein (AFP) levels were lower on average in 14 pregnancies with Down syndrome tested at 16.5 to 17.5 weeks' gestation than in 22 pregnancies tested at 14 to 16.5 weeks and in 15 at 17.5 to 21.5 weeks. Expressing levels in multiples of the median for unaffected pregnancies of the same gestation, the mean value at each gestation was 0.59, 0.74, and 0.69 respectively. However, these differences are not borne out by summarizing other published studies.

Table I updates a previous summary of the literature[1] to include all recent publications[2-5] in which individual multiple of the median values according to week of gestation are either tabulated or can be abstracted from a figure and where the serum sample was not taken after amniocentesis or chorionic villus biopsy. A total of 379 affected pregnancies between 14 and 21 weeks were available for analysis. There was no tendency for the median AFP level in any gestational week to differ from the overall median value, nor did any effect of gestation emerge when an analysis of variance of log AFP was carried out to allow for differences between the various studies included. First-trimester AFP levels have been reported in a total of 67 pregnancies with Down syndrome[6-10] and found to be similar to second-trimester levels. The geometric mean weighted for the number of pregnancies in each first-trimester study was 0.74 multiples of the median.

Table I. Maternal serum AFP levels in pregnancies with fetal syndrome according to gestational age[1-5]

Gestation completed (wk)	*No. of pregnancies*	*Median AFP (MoM)*
14	2	0.72-0.73
15	26	0.72
16	144	0.72
17	110	0.72
18	53	0.70
19	29	0.74
20	13	0.77
21	2	0.45-1.96
14-21	379	0.72

MoM, Multiple of the median for unaffected pregnancies of the same gestation.

These results have implications for screening policy in centers in which AFP is the only Down syndrome marker being measured. If the same blood sample is used for both Down syndrome and neural tube defect screening the test would need to be done at 16 to 18 weeks, the gestation at which neural tube defect detection is highest. If a separate blood sample can be used or if reliance can be placed on ultrasonography for neural tube defect screening, Down syndrome screening need not be restricted to 16 to 18 weeks. For centers using multiple markers the optimal gestation will depend on which additional markers are used.

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Reply

To the Editors: We are pleased to have this opportunity to emphasize the importance of pursuing improvements in the efficacy of the maternal serum α-fetoprotein (AFP) screening test for Down syndrome pregnancies. Comparing affected with unaffected pregnancies at our center we found that for a given maternal serum AFP cut point of 0.8 weight-adjusted multiples of the median the detection rate was higher and the false-positive rate was lower between 16.5 and 17.5 weeks' gestation than at earlier or later gestational ages. This suggested to us that the optimum gestational age for maternal serum AFP screening for Down syndrome may be similar to the optimum reported previously for neural tube defect screening.[1] Wald and Cuckle's[2] previous and current synthesis of maternal serum AFP multiples of the median data from different centers focus on mean or median values in Down syndrome pregnancies. Such values relate only to detection rates and only indirectly so. Although Cuckle did not detect statistically significant differences among the gestational age–specific median maternal serum AFP multiples of the median he reported, it is interesting to note that a nadir of 0.7 multiples of the median was reported at 17 weeks' gestation in his first paper[2] and at 18 weeks' gestation in his updated table. It is possible that the nadir between 17 and 18 weeks' gestation is real, not a result of random fluctuation, and that it implies a higher detection rate for Down syndrome pregnancies in that time window. A similar pattern of maternal serum AFP multiples of the median results (with a nadir at 17 weeks' gestation) was reported by Waller et al.[3] To assess accurately whether the patterns observed by Waller and Cuckle suggest underlying detection rates and false-positive rates similar to ours would require comparable analyses allowing for variance in unaffected and affected pregnancies. We urge individual centers to periodically examine their maternal serum AFP detection rates and false-positive rates in this manner. Given the quantitative advantages and power of recent metaanalysis techniques[4] to properly incorporate center-specific variances, we recommend the application of these methods to assist policy decisions in maternal serum AFP screening. Finally, we stress the importance of appropriate tests for heterogeneity among centers before any pooling of data across centers. In assessing heterogeneity as in pooling across centers, maternal serum AFP variability in both unaffected and affected pregnancies must be taken into consideration.

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An association between placental location and nuchal cord occurrence

To the Editors: A continuing prospective study named the Perinatal Umbilical Cord Project made an association between placental location and nuchal cord occurrence.[1] The number of entries into this private study has now numbered 400 cases. The purpose of this letter is to update the statistics on nuchal cord and placental location.

Four hundred pregnancies were prospectively studied over a 3-year period. Three hundred sixty met requirements of a 28-week ultrasonographic examination and manual removal of the placenta at delivery. Placental location was reviewed after delivery and compared with the occurrence of nuchal cord. Nuchal cord is defined as the umbilical cord being 360 degrees around the neck.

Table I summarizes the data.

Among 360 pregnancies the location of the placenta was recorded as follows: posterior, 37%; anterior, 38%; and fundal, 25%. The occurrence of a nuchal cord (14%) among all pregnancies is not the same for each of the placental locations; it is most common among

Table I. Summary of data

Placental location	*n*	*Nuchal cord*	*Percentage with nuchal cord*
Posterior	134	30	22.4
Anterior	137	16	11.7
Fundal	89	4	4.5
TOTAL	360	50	13.9

posterior positions (22.4%), nearly twice that for anterior positions (11.7%), and five times that for fundal location (4.5%). These differences in incidence are statistically significant (logistic regression $p = 0.0003$). The posterior placenta has a statistically significant association with nuchal cord versus other placental locations.

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Menopausal patient considerations

To the Editors: The article by Sutton et al. (Sutton GP, Bundy BN, Delgado G, et al. Ovarian metastases in stage IB carcinoma of the cervix: a gynecologic oncology group study. Am J Obstet Gynecol 1992:166:50-3) presents an interesting subset of data collected as part of a large surgical staging protocol by the Gynecologic Oncology Group. The data focus on the overall incidence of ovarian metastases in patients with stage IB cervical cancer. It would be most helpful if the authors would further review their data to focus on the incidence of occult ovarian metastases in patients believed to be candidates for ovarian preservation at the time of radical hysterectomy and bilateral pelvic node dissection for early cervical cancer.

Of their 1125 patients, 135 (12%) were excluded for protocol problems and an additional 111 (11.2%) did not undergo radical hysterectomy with bilateral salpingo-oophorectomy because of extensive disease found at surgery. Of the remaining 879 patients 833 underwent radical hysterectomy (I cannot account for the other 46 patients); of the 833, 439 had a radical hysterectomy with bilateral salpingo-oophorectomy.

Of these 439 women, the authors could delete those who were postmenopausal (or >40 years old?) and those in whom incidental ovarian pathologic conditions were found. The remaining patients would be those in whom some physicians would consider ovarian preservation at the time of radical hysterectomy. The incidence of occult metastatic disease for various cell types could then be calculated to help allay our fears that ovarian preservation during surgery for early adenocarcinoma of the cervix is not as safe as has been established for squamous lesions.

Tubata et al.[1] reported that 2 (77%) of 26 cases of stage IB adenocarcinoma of the cervix had ovarian metastases; this statistic heightens our concerns.

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Response declined

Daily commuting and preterm birth rate

To the Editors: In a survey carried out in two maternity units in the Paris region (1987 through 1988) we studied risk factors of preterm births among working women. The sample included 838 women who were interviewed during their stay in the hospital after delivery; among them, 61% had jobs during their pregnancy. Women who had a qualified job in professional, intermediate, or clerical occupations had a significantly lower rate of preterm births than women with less-qualified jobs in industrial, service, and shop occupations (Table I). This finding agrees with the literature.[1-3]

In addition, women whose daily commuting time was ≥2 hours with public transportation had a preterm birth rate of >2 times that of women whose commuting time on public transportation was shorter or women who used other types of transportation such as their own cars or walking. This difference appeared clearly in both groups of occupations; adjustment for occupational group was a way to control roughly for social characteristics and working conditions.[3] Women who used public transportation for <2 hours daily did not have an excess of preterm births. In our sample 33% were exposed to ≥2 hours of public transportation each day.

Kaminski and Papiernik[4] had already shown that long daily travel had to be included in a score of prediction of preterm birth from data collected in a ma-

Table I. Preterm birth rate and daily commuting in the Paris region

	Preterm births					
	All women		*Public transportation ≥2 hours*		*Other*	
	No.	*%*	*No.*	*%*	*No.*	*%*
Working women						
Qualified jobs	367	5	115	9	235	4
Less-qualified jobs	127	11	33	18	85	9
TOTAL	494	7	148	11	320	5 $p < 0.05$*

*Mantel-Haenszel χ^2 test adjusted for qualification of jobs.

ternity ward in Paris in 1969. Other French surveys conducted later on national samples or on regional samples[1] outside of Paris did not report any clear relationship between daily travels and the preterm birth rate.

Long daily travels are a consequence of the development of very large cities, which depend on urban policy and the labor market. Even if it is important that women be aware of the risks, it is probably unnecessary to recommend that they change their commuting habits. The mode of transportation they have chosen is the result of a compromise between several personal factors, and for them it is the only feasible solution. Doctors have to keep in mind that long commuting may be a risk factor for preterm birth, and all decision-makers must be aware of the health consequences of their economic choices.

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Imaging of the levonorgestrel implantable contraceptive device

To the Editors: The availability of the levonorgestrel implantable contraceptive device in the United States has led to its frequent use and predictions that many users of oral contraceptives will switch to the levonorgestrel implant device (Norplant, Wyeth-Ayerst, Philadelphia).[1]

The routine removal of such a device should pose no problem in the vast majority of cases because the subcutaneous implants are palpable. However, in cases in which infection develops or the implant migrates to a deeper nonpalpable location, the identification and removal of implants may be a problem. Prompted by such a case, we attempted to identify the levonorgestrel implantable contraceptive device in a normal location with roentgenography and ultrasonography of the arm.

The implant was easily identified on an anterior-posterior projection film of the distal humerus with soft-tissue film and filming technique (55 kvp, 10 mA sec). The six fanlike projections of the levonorgestrel device are radiopaque (Fig. 1). Nonpalpable implants could be easily localized with radiopaque skin markers similar to those techniques used in mammography.

With a 10 MHz transducer and 1 cm gel pad the six arms of the levonorgestrel implant device can be identified in the transverse plane on the basis of their posterior acoustic shadowing, a characteristic often seen with high-attenuation foreign bodies (Fig. 2). The longitudinal axis can also be identified because of the posterior shadowing.

Plain film roentgenography of the arm for localiza-



Fig. 1. Soft-tissue x-ray film of the forearm shows the levonorgestrel implantable device with the fan-like configuration of its six radiopaque arms *(arrows)*.

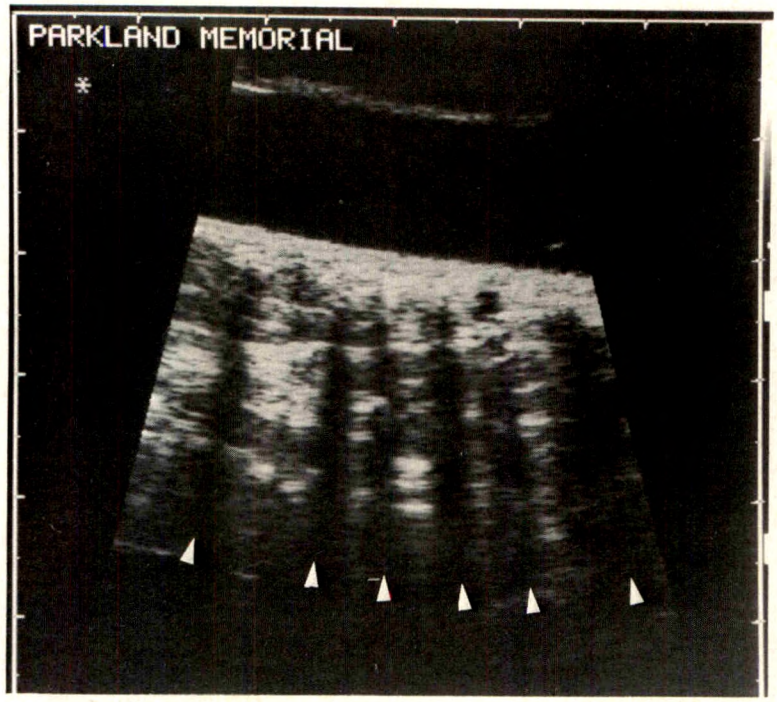


Fig. 2. Ultrasonogram of the levonorgestrel implantable device with the six arms in their axial planes shows the posterior shadowing from the high attenuation of the implant *(arrowheads)*.

tion in our case was faster, easier, less expensive; it also provided better resolution than ultrasonography. Localization for nonpalpable implants can be easily done with skin markers.

In summary, a single anterioposterior soft-tissue roentgenography of the arm can localize the radiopaque levonorgestrel implant device easily and with favorable resolution. This is recommended when palpable localization of the levonorgestrel implant device is not possible.

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Color Atlas and Synopsis of Sexually Transmitted Diseases. Edited by H. Hunter Handsfield. 203 pages, illustrated. New York, 1992, McGraw-Hill Inc. $35.

Colposcopy-Cervical Pathology. Textbook and Atlas. Edited by Erich Burghardt. 284 pages, illustrated. New York, 1992, Thieme Medical Publishers, Inc. No price listed.

Comprehensive Gynecology. Second edition. Edited by Arthur L. Herbst, Daniel R. Mishell, Jr., Morton A. Stenchever, and William Droegemueller. 1334 pages, illustrated. St. Louis, 1992, Mosby–Year Book, Inc. No price listed.

Comprehensive Gynecology Review. Second edition. Edited by Holzman, Ling, Laube, and Vontver. 298 pages, illustrated. St. Louis, 1992, Mosby–Year Book, Inc. No price listed.

Current Medical Diagnosis and Treatment. Edited by Steven A. Schroeder, Lawrence M. Tierney, Stephen J. McPhee, Maxine A. Papadakis, and Marcus A. Krupp. 1281 pages, illustrated. Norwalk, Connecticut, 1992, Appleton & Lange. $39.95.

Diagnosing Male Infertility. New Possibilities and Limits. Edited by G.M. Colpi and D. Pozza. 250 pages, illustrated. Basel, Switzerland, 1992, Karger. $197 (U.S.).

Diagnostic Imaging in Infertility. Second edition. Edited by Alan C. Winfield and Anne Colston Wentz. 312 pages, illustrated. Baltimore, 1992, Williams & Wilkins. $85.

Disorders of the Placenta, Fetus, and Neonate. Diagnosis and Clinical Significance. Edited by Richard L. Naeye. 375 pages, illustrated. St. Louis, 1992, Mosby–Year Book, Inc. $75.

Drug Information for the Health Care Professional, Volumes IA and IB. Advice for the Patient, Volume II. Approved Drug Products and Legal Requirements, Volume III. Twelfth edition. Edited by The United States Pharmacopeial Convention Inc. Kingsport, Tennessee, 1992, printed by Arcata Graphics. $200 (complete set).

Drug Therapy in Obstetrics and Gynecology. Third edition. Edited by William F. Rayburn and Frederick P. Zuspan. 661 pages, with a Quick Reference to Drug Therapy in OB/GYN pamphlet. St. Louis, 1992, Mosby–Year Book, Inc. No price listed.

Fetal and Perinatal Neurology. Edited by Yukio Fukuyama, Yoshiyuki Suzuki, Shigehiko Kamoshita, and Paul Casaer. 388 pages. Basel, Switzerland, 1992, S. Karger AG. $232.

Fetal Neural Development and Adult Schizophrenia. Edited by Sarnoff A. Mednick, Tyrone D. Cannon, Christopher E. Barr, and Melvin Lyon. 244 pages. New York, 1991, Cambridge University Press. $89.95.

Heredity and Your Family's Health. Edited by Aubrey Milunsky. 512 pages. Baltimore, 1992, Johns Hopkins University Press. $18.95.

HIV Counselling. Second edition. Edited by Tom Bond. 100 pages. Cambridge, England, United Kingdom, 1992, Daniels Publishing. No price listed.

Illustrated Textbook of Gynaecology. Second edition. Edited by Eric V. Mackay, Norman A. Beischer, Roger J. Pepperell, and Carl Wood. 624 pages, illustrated. Philadelphia, 1992, W.B. Saunders. $49.

In Sickness and in Health: What Every Man Should Know About the Woman He Loves. Edited by Mary E. O'Brien. 208 pages, illustrated. Santa Fe, 1991, Health Press. $14.95.

Integrated Colposcopy. A Text and Atlas. Edited by Malcolm Anderson, Joe Jordan, Anne Morse, and Frank Sharp. 235 pages, illustrated. St. Louis, 1991, Mosby–Year Book, Inc. No price listed.

The Joy of Uncircumcising. Restore Your Birthright and Maximize Sexual Pleasure! Edited by Jim Bigelow. 239 pages. Oakland, California, 1992, Hourglass Book Publishing. $16.95.

Love Cycles. The Science of Intimacy. Edited by Winifred B. Cutler. 331 pages. New York, 1991, Villard Books. No price listed.

Manual of Outpatient Gynecology. Second edition. Edited by Carl S. Havens, Nancy D. Sullivan, Patti Tilton. 301 pages. Boston, 1991, Little, Brown and Company. $23.50.

Maternity Ward: Behind the Scenes at a Big City Hospital. Edited by Susan Stanley. 303 pages. New York, 1992, William Morrow and Company, $22.

Menopause. A Guide for Women and the Men Who Love Them. Revised edition. Edited by Winnifred B. Cutler and Celso-Ramon Garcia. 431 pages. New York, 1992, W.W. Norton and Company. $25.

Menstrual Health in Women's Lives. Edited by Alice J. Dan and Linda L. Lewis. 301 pages. Chicago, 1992, University of Illinois Press. $37.50 (cloth), $16.95 (paperback).

Micromanipulation of Human Gametes and Embryos. Edited by Jacques Cohen, Henry E. Malter, Beth E. Talansky, and Jamie Grifo. 336 pages, illustrated. New York, 1992, Raven Press. $89.

Modern Perspectives of Child Neurology. Edited by Yukio Fukuyama, Shigehiko Kamoshita, Chikaya Ohtsuka, and Yoshiyuki Suzuki. 360 pages. Tokyo, 1991, Japanese Society of Child Neurology. No price listed,

Oral Contraceptives and Breast Cancer. Edited by Institutes of Medicine. 185 pages. Washington D.C., 1991, National Academy Press. $24.95.

Oxford Handbook of Clinical Specialties. Third edition. Edited by J.A.B. Collier, J.M. Longmore, and J.H. Harvey. 811 pages. New York, 1991, Oxford University Press. $27.50 (cloth).

Pediatric and Adolescent Gynecology. Edited by Sue Ellen Koehler Carpenter and John A. Rock. 493 pages. New York, 1992, Raven Press. $69.

Placenta: Basic Research for Clinical Research for Clinical Application. Edited by H. Soma. 214 pages, illustrated. Basel, Switzerland, 1991, S. Karger AG. $184 (U.S.).

Pregnancy, Diabetes and Birth. A Management Guide. Edited by Dorothy Reycroft Hollingsworth. 288 pages, illustrated. Baltimore, 1992, Williams & Wilkins. $70.

Ready to Learn. A Mandate for the Nation. The Carnegie Foundation for the Advancement of Teaching. Edited by Ernest L. Boyer. 193 pages. Lawrenceville, New Jersey, 1991, Princeton University Press. $8.

Reproduction, Growth and Development: Serono Symposia Publications from Raven Press. Volume 71. Edited by A. Negro-Vilar and G. Perez-Palacios. 456 pages. New York, 1992, Raven Press. $130.

Say No to Circumcision. Edited by Thomas J. Ritter. 128 pages. Oakland, California, 1992, Hourglass Book Publishing. $10.95.

Surgical Obstetrics. Edited by Warren C. Plauche, John C. Morrison, and Mary Jo O'Sullivan. 534 pages, illustrated. Philadelphia, 1992, W.B. Saunders Company. $85.

Teaching Atlas of Breast Ultrasound. Edited by Wolfgang Leucht. 224 pages, illustrated. New York, 1992, Thieme Medical Publishers. No price listed.

When a Baby Dies? Psychotherapy for Pregnancy and Newborn Loss. Edited by Irving G. Leon. 230 pages. New Haven, Connecticut, 1992, Yale University Press. $12 (paperback), $27.50 (cloth).

When Did I Begin? Conception of the Human Individual in History, Philosophy and Science. Edited by Norman M. Ford. 217 pages. New York, 1991, Cambridge University Press. $16.95 (paperback).

Which Tests for my Unborn Baby? Edited by Lachlan de Crespigny and Rhonda Dredge. 170 pages, illustrated. New York, 1991, Oxford University Press. $19.95.

Correction

In the article by Wild, Alaupovic, and Parker entitled "Lipid and apolipoprotein abnormalities in hirsute women" (AM J OBSTET GYNECOL 1992;166:1191-7), on page 1194, Fig. 1 relates to the Discussion by Dr. Brian A. Little and should have appeared with that Discussion on page 1196. The legend should have included the following information: (Morris D, et al. [Abstract]. In: Proceedings of the thirty-seventh annual meeting of the Canadian Fertility and Andrology Society, 1991:55.)

ITEMS

Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $60.00 U.S. per insertion and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to Kay G. Goehler, Senior Manuscript Editor, Journal Editing, Mosby–Year Book, Inc., 11830 Westline Industrial Drive, St. Louis, MO 63146-3318.

The Thomas Garite/Roger Freeman Obstetrical Symposium, October 23 and 24, 1992, Claremont Resort, Berkeley, California. Sponsored by Alta Bates Medical Center. For information contact: Mary Grim, Medical Education Program Manager, tel.: (510) 204-1420.

National Perinatal Association 1992 Annual Clinical Conference and Exposition, "Vision of the Future: Changes and Challenges in Perinatal Care," November 19-22, 1992, Buena Vista Palace, Orlando, Florida. For complete conference brochure, abstract application, or exhibitor information call NPA at (813) 971-1008 or Contemporary Forums at (510) 828-7100, ext. 102.

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November 16-21, 1992, 15th Annual OB/GYN Review, Costa Mesa, California

January 13-17, 1993, 8th Annual Perinatal Symposium, Beaver Creek, Oregon

February 27–March 6, 1993, 14th Annual Current Topics in OB, Aspen, Colorado

March 25-27, 1993, Gynecologic Surgery, Rancho Las Palmas, California

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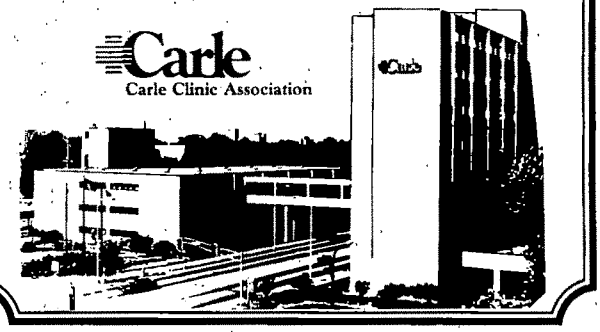
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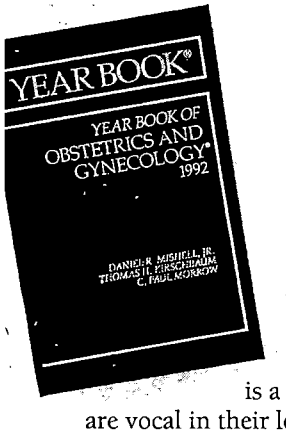
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INDICATIONS AND USAGE: PREVENTION OF PREGNANCY.

CONTRAINDICATIONS: Oral contraceptives should not be used in women who currently have the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophlebitis or thromboembolic disorders. 3. Cerebral vascular or coronary artery disease. 4. Known or suspected carcinoma of the breast. 5. Carcinoma of the endometrium or other known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Cholestatic jaundice of pregnancy or jaundice with prior pill use. 8. Hepatic adenomas or carcinomas. 9. Known or suspected pregnancy.

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risks of several serious conditions including myocardial infarction, thromboembolism, stroke, hepatic neoplasia, and gallbladder disease, although the risk of serious morbidity or mortality is very small in healthy women without underlying risk factors. The risk of morbidity and mortality increases significantly in the presence of other underlying risk factors such as hypertension, hyperlipidemias, obesity and diabetes. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks. The information contained in this brief summary is principally based on studies carried out in patients who used oral contraceptives with higher formulations of estrogens and progestogens than those in common use today. The effect of long term use of the oral contraceptives with lower formulations of both estrogens and progestogens remains to be determined. Throughout this brief summary epidemiological studies reported are of two types: retrospective or case control studies and prospective or cohort studies. Case control studies provide a measure of the relative risk of a disease, namely a *ratio* of the incidence of a disease among oral contraceptive users to that among nonusers. The relative risk does not provide information on the actual clinical occurrence of a disease. Cohort studies provide a measure of attributable risk, which is the *difference* in the incidence of disease between oral contraceptive users and nonusers. The attributable risk does provide information about the actual occurrence of a disease in the population. For further information, the reader is referred to a text on epidemiological methods. 1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS. a. Myocardial Infarction. An increased risk of myocardial infarction has been associated with oral contraceptive use. This risk is primarily in smokers or women with other underlying risk factors for coronary artery disease such as hypertension, hypercholesterolemia, morbid obesity, and diabetes. The relative risk of heart attack for current oral contraceptive users has been estimated to be two to six. The risk is very low under the age of 30. Smoking in combination with oral contraceptive use has been shown to contribute substantially to the incidence of myocardial infarctions in women in their mid-thirties or older with smoking accounting for the majority of excess cases. Mortality rates associated with circulatory disease have been shown to increase substantially in smokers, especially in those 35 years of age and older among women who use oral contraceptives. Oral contraceptives may compound the effects of well-known risk factors, such as hypertension, diabetes, hyperlipidemias, age and obesity. In particular, some progestogens are known to decrease HDL cholesterol and cause glucose intolerance, while estrogens may create a state of hyperinsulinism. Oral contraceptives have been shown to increase blood pressure among users (see section 9 in WARNINGS). Similar effects on risk factors have been associated with an increased risk of heart disease. Oral contraceptives must be used with caution in women with cardiovascular disease risk factors. b. Thromboembolism. An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Case control studies have found the relative risk of users compared to non-users to be 3 for the first episode of superficial venous thrombosis, 4 to 11 for deep vein thrombosis or pulmonary embolism, and 1.5 to 6 for women with predisposing conditions for venous thromboernbolic disease. Cohort studies have shown the relative risk to be somewhat lower, about 3 for new cases and about 4.5 for new cases requiring hospitalization. The risk of thromboembolic disease associated with oral contraceptives is not related to length of use and disappears after pill use is stopped. A two- to four-fold increase in relative risk of post-operative thromboembolic complications has been reported with the use of oral contraceptives. The relative risk of venous thrombosis in women who have predisposing conditions is twice that of women without such medical conditions. If feasible, oral contraceptives should be discontinued at least four weeks prior to and for two weeks after elective surgery of a type associated with an increase in risk of thromboembolism and during and following prolonged immobilization. Since the immediate postpartum period is also associated with an increased risk of thromboembolism, oral contraceptives should be started no earlier than four weeks after delivery in women who elect not to breast feed. c. Cerebrovascular diseases. Oral contraceptives have been shown to increase both the relative and attributable risks of cerebrovascular events (thrombotic and hemorrhagic strokes), although, in general, the risk is greatest among older (>35 years), hypertensive women who also smoke. Hypertension was found to be a risk factor for both users and non-users, for both types of strokes, and smoking interacted to increase the risk of stroke. In a large study, the relative risk of thrombotic strokes has been shown to range from 3 for normotensive users to 14 for users with severe hypertension. The relative risk of hemorrhagic stroke is reported to be 1.2 for nonsmokers who used oral contraceptives, 2.6 for smokers who did not use oral contraceptives, 7.6 for smokers who used oral contraceptives, 1.8 for normotensive users and 25.7 for users with severe hypertension. The attributable risk is also greater in older women. d. Dose-related risk of vascular disease from oral contraceptives. A positive association has been observed between the amount of estrogen and progestogen in oral contraceptives and the risk of vascular disease. A decline in serum high density lipoproteins (HDL) has been reported with many progestational agents. A decline in serum high density lipoproteins has been associated with an increased incidence of ischemic heart disease. Because estrogens increase HDL cholesterol, the net effect of an oral contraceptive depends on a balance achieved between doses of estrogen and progestogen and the activity of the progestogen used in the contraceptive. The activity and amount of both hormones should be considered in the choice of an oral contraceptive. Minimizing exposure to estrogen and progestogen is in keeping with good principles of therapeutics. For any particular estrogen/progestogen combination, the dosage regimen prescribed should be one which contains the least amount of estrogen and progestogen that is compatible with a low failure rate and the needs of the individual patient. New acceptors of oral contraceptive agents should be started on preparations containing 0.035 mg or less of estrogen. e. Persistence of risk of vascular disease. There are two studies which have shown persistence of risk of vascular disease for ever-users of oral contraceptives. In a study in the United States, the risk of developing myocardial infarction after discontinuing oral contraceptives persists for at least 9 years for women 40-49 years who had used oral contraceptives for five or more years, but this increased risk was not demonstrated in other age groups. In another study in Great Britain, the risk of developing cerebrovascular disease persisted for at least 6 years after discontinuation of oral contraceptives, although excess risk was very small. However, both studies were performed with oral contraceptive formulations containing 50 micrograms or higher of estrogens. 2. ESTIMATES OF MORTALITY FROM CONTRACEPTIVE USE. One study gathered data from a variety of sources which have estimated the mortality rate associated with different methods of contraception at different ages. These estimates include the combined risk of death associated with contraceptive methods plus the risk attributable to pregnancy in the event of method failure. Each method of contraception has its specific benefits and risks. The study concluded that with the exception of oral contraceptive users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of birth control is low and below that associated with childbirth. The observation of an increase in risk of mortality with age for oral contraceptive users is based on data gathered in the 1970's. Current clinical recommendation involves the use of lower estrogen dose formulations and a careful consideration of risk factors. In 1989, the Fertility and Maternal Health Drugs Advisory Committee was asked to review the use of oral contraceptives in women 40 years of age and over. The Committee concluded that although cardiovascular disease risks may be increased with oral contraceptive use after age 40 in healthy non-smoking women (even with the newer low-dose formulations), there are also greater potential health risks associated with pregnancy in older women and with the alternative surgical and medical procedures which may be necessary if such women do not have access to effective and acceptable means of contraception. The Committee recommended that the benefits of low-dose oral contraceptive use by healthy non-smoking women over 40 may outweigh the possible risks. Of course, older women, as all women who take oral contraceptives, should take an oral contraceptive which contains the least amount of estrogen and progestogen that is compatible with a low failure rate and individual patient needs. 3. CARCINOMA OF THE REPRODUCTIVE ORGANS. Numerous epidemiological studies have been performed on the incidence of breast, endometrial, ovarian and cervical cancer in women using oral contraceptives. While there are conflicting reports most studies suggest that use of oral contraceptives is not associated with an overall increase in the risk of developing breast cancer. Some studies have reported an increased relative risk of developing breast cancer, particularly at a younger age. This increased relative risk appears to be related to duration of use. Some studies suggest that oral contraceptive use has been associated with an increase in the risk of cervical intraepithelial neoplasia in some populations of women. However, there continues to be controversy about the extent to which such findings may be due to differences in sexual behavior and other factors. 4. HEPATIC NEOPLASIA. Benign hepatic adenomas are associated with oral contraceptive use, although the incidence of benign tumors is rare in the United States. Indirect calculations have estimated the attributable risk to be in the range of 3.3 cases/100,000 for users, a risk that increases after four or more years of use especially with oral contraceptives of higher dose. Rupture of benign, hepatic adenomas may cause death through intra-abdominal hemorrhage. Studies from Britain have shown an increased risk of developing hepatocellular carcinoma in long-term (>8 years) oral contraceptive users. However, these cancers are rare in the U.S. and the attributable risk (the excess incidence) of liver cancers in oral contraceptive users approaches less than one per million users. 5. OCULAR LESIONS. There have been clinical case reports of retinal thrombosis associated with the use of oral contraceptives. Oral contraceptives should be discontinued if there is unexplained partial or complete loss of vision; onset of proptosis or diplopia, papilledema, or retinal vascular lesions. Appropriate diagnostic and therapeutic measures should be undertaken immediately. 6. ORAL CONTRACEPTIVE USE BEFORE OR DURING EARLY PREGNANCY. Extensive epidemiological studies have revealed no increased risk of birth defects in women who have used oral contraceptives prior to pregnancy. The majority of recent studies also do not indicate a teratogenic effect, particularly in so far as cardiac anomalies and limb reduction defects are concerned, when taken inadvertently during early pregnancy. The administration of oral contraceptives to induce withdrawal bleeding should not be used as a test for pregnancy. Oral contraceptives should not be used during pregnancy to treat threatened or habitual abortion. It is recommended that for any patient who has missed two consecutive periods pregnancy should be ruled out before continuing oral contraceptive use. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period. Oral contraceptive use should be discontinued until pregnancy is ruled out. 7. GALLBLADDER DISEASE. Earlier studies have reported an increased lifetime relative risk of gallbladder surgery in users of oral contraceptives and estrogens. More recent studies, however, have shown that the relative risk of developing gallbladder disease among oral contraceptive users may be minimal. The recent findings of minimal risk may be related to the use of oral contraceptive formulations containing lower hormonal doses of estrogens and progestogens. 8. CARBOHYDRATE AND LIPID METABOLIC EFFECTS. Oral contraceptives have been shown to cause a decrease in glucose tolerance in a significant percentage of users. This effect has been shown to be directly related to estrogen dose. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents. However, in the non-diabetic woman, oral contraceptives appear to have no effect on fasting blood glucose. Because of these demonstrated effects prediabetic and diabetic women in particular should be carefully monitored while taking oral contraceptives. A small proportion of women will have persistent hypertriglyceridemia while on the pill. As discussed earlier (see WARNINGS 1a and 1d), changes in serum triglycerides and lipoprotein levels have been reported in oral contraceptive users. 9. ELEVATED BLOOD PRESSURE. An increase in blood pressure has been reported in women taking oral contraceptives, and this increase is more likely in older oral contraceptive users and with extended duration of use. Data from the Royal College of General Practitioners and subsequent randomized trials have shown that the incidence of hypertension increases with increasing progestational activity. Women with a history of hypertension or hypertension-related diseases, or renal disease, should be encouraged to use another method of contraception. If women elect to use oral contraceptives, they should be monitored closely and if significant elevation of blood pressure occurs, oral contraceptives should be discontinued. For most women, elevated blood pressure will return to normal after stopping oral contraceptives, and there is no difference in the occurrence of hypertension between former and never users. 10. HEADACHE. The onset or exacerbation of migraine or development of headache with a new pattern which is recurrent, persistent or severe requires discontinuation of oral contraceptives and evaluation of the cause. 11. BLEEDING IRREGULARITIES. Breakthrough bleeding and spotting are sometimes encountered in patients on oral contraceptives, especially during the first three months of use. Non-hormonal causes should be considered and adequate diagnostic measures taken to rule out malignancy or pregnancy in the event of breakthrough bleeding as in the case of any abnormal vaginal bleeding. If pathology has been excluded, time or a change to another formulation may solve the problem. In the event of amenorrhea, pregnancy should be ruled out. Some women may encounter post-pill amenorrhea or oligomenorrhea, especially when such a condition was preexistent. 12. ECTOPIC PREGNANCY. Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. **PRECAUTIONS:** 1. PHYSICAL EXAMINATION AND FOLLOW UP. A complete medical history and physical examination should be taken prior to the initiation or reinstitution of oral contraceptives and at least annually during use of oral contraceptives. These physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including cervical cytology, and relevant laboratory tests. In case of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be conducted to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care. 2. LIPID DISORDERS. Women who are being treated for hyperlipidemias should be followed closely if they elect to use oral contraceptives. Some progestogens may elevate LDL levels and may render the control of hyperlipidemias more difficult. 3. LIVER FUNCTION. If jaundice develops in any woman receiving such drugs, the medication should be discontinued. Steroid hormones may be poorly metabolized in patients with impaired liver function. 4. FLUID RETENTION. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention. 5. EMOTIONAL DISORDERS. Women with a history of depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. 6. CONTACT LENSES. Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist. 7. DRUG INTERACTIONS. Reduced efficacy and increased incidence of breakthrough bleeding and menstrual irregularities have been associated with concomitant use of rifampin. A similar association, though less marked, has been suggested with barbiturates, phenylbutazone, phenytoin sodium, and possibly with griseofulvin, ampicillin and tetracyclines. 8. INTERACTIONS WITH LABORATORY TESTS. Certain endocrine and liver function tests and blood components may be affected by oral contraceptives: a. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3, increased norepinephrine-induced platelet aggregability. b. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or by radio-immunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. c. Other binding proteins may be elevated in serum. d. Sex-binding globulins are increased and result in elevated levels of total circulating sex steroids and corticoids; however, free or biologically active levels remain unchanged. e. Triglycerides may be increased. f. Glucose tolerance may be decreased. g. Serum folate levels may be depressed by oral contraceptive therapy. This may be of clinical significance if a woman becomes pregnant shortly after discontinuing oral contraceptives. 9. CARCINOGENESIS. See WARNINGS section. 10. PREGNANCY. Pregnancy Category X. See CONTRAINDICATIONS and WARNINGS sections. 11. NURSING MOTHERS. Small amounts of oral contraceptive steroids have been identified in the milk of nursing mothers and a few adverse effects on the child have been reported, including jaundice and breast enlargement. In addition, oral contraceptives given in the postpartum period may interfere with lactation by decreasing the quantity and quality of breast milk. If possible, the nursing mother should be advised not to use oral contraceptives but to use other forms of contraception until she has completely weaned her child. **INFORMATION FOR THE PATIENT: See Patient Package Insert.**

ADVERSE REACTIONS: An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS section). Thrombophlebitis and venous thrombosis with or without embolism. Arterial thromboembolism. Pulmonary embolism. Myocardial infarction. Cerebral hemorrhage. Cerebral thrombosis. Hypertension. Gallbladder disease. Hepatic adenomas or benign liver tumors. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea. Vomiting. Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding. Spotting. Change in menstrual flow. Amenorrhea. Temporary infertility after discontinuation of treatment. Edema. Melasma which may persist. Breast changes: tenderness, enlargement, secretion. Change in weight (increase or decrease). Change in cervical erosion and secretion. Diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives and the association has been neither confirmed nor refuted: Pre-menstrual syndrome. Cataracts. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiforme. Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. Hemolytic uremic syndrome. Acne. Changes in libido. Colitis.

OVERDOSAGE: Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea and withdrawal bleeding may occur in females.

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Serious as well as minor side effects have been reported with the use of oral contraceptives. The physician should remain alert to the earliest manifestations of any symptoms of serious disease and discontinue oral contraceptive therapy when appropriate.

Please see complete Prescribing Information, a brief summary of which is on the following page.

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